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Concentrations of strontium in the pectoral fin rays of the white sturgeon (*Acipenser transmontanus*) by laser ablation sampling – inductively coupled plasma – mass spectrometry as an indicator of marine migrations

Geoff Veinott, Tom Northcote, Marvin Rosenau, and R. Douglas Evans

Abstract: Laser ablation sampling – inductively coupled plasma – mass spectrometry (LAS-ICP-MS) was an effective technique for the comparison of relative Sr concentrations in the opaque growth zones (annuli) in the fin rays of white sturgeon (*Acipenser transmontanus*). Three of 29 Fraser River white sturgeon (10.3% of the sample) showed significantly higher Sr fin ray concentrations in at least one annulus compared with the remainder of the fin ray, and this was interpreted as evidence of marine migrations. Eleven other individual fish had significantly higher mean Sr concentrations in their first 15 annuli compared with annuli 16–30 but lower than the concentrations of the three putative diadromous fish, and this was interpreted as evidence of time spent in the estuary. Eight Fraser River fish greater than 15 years old showed no significant increase in fin ray Sr concentations in the first 15 annuli, and Sr concentrations remained below 350 ppm throughout their lives. These individuals were considered likely to have spent most of their lives in a freshwater environment. Based on the evidence in this study, it is probable that the majority of white sturgeon in the lower Fraser River are not diadromous, but many spend extended periods of time in the Fraser River estuary as juveniles.

Résumé : La spectrométrie de masse à plasma induit par haute fréquence – échantillonnage par ablation laser s'est révélée une technique efficace de comparaison des concentrations relatives de Sr dans les zones de croissance opaques (anneaux) des rayons des nageoires chez l'esturgeon blanc (*Acipenser transmontanus*). Chez trois des 29 esturgeons blancs (10,3% de l'échantillon) du Fraser, on a relevé des concentrations significativement plus élevées de Sr dans les rayons des nageoires dans au moins un anneau par rapport au reste du rayon, ce qui a été interprété comme une preuve de migration en mer. Chez onze autres esturgeons, les concentrations moyennes de Sr étaient significativement plus élevées dans les quinze premiers anneaux comparativement aux anneaux 16–30, mais plus faibles que les concentrations mesurées chez les trois poissons qui semblent diadromes; ces données ont été interprétées comme une indication de temps passé dans l'estuaire. Huit poissons du Fraser âgés de plus de 15 ans ne présentaient aucune augmentation importante de la concentration de Sr dans les quinze premiers anneaux des rayons, et la concentration de Sr est demeurée inférieure à 350 ppm pendant toute leur vie. Ces individus ont été considérés comme ayant passé probablement presque toute leur vie en eau douce. D'après les indications de la présente étude, la majorité des esturgeons blancs du cours inférieur du Fraser ne seraient pas diadromes, mais un grand nombre passent de longues périodes dans l'estuaire du Fraser à l'état juvénile.

[Traduit par la Rédaction]

Introduction

In recent years, there has been much worldwide public and scientific interest in the various species of sturgeon (Acipenseridae) and their dramatic declines in population numbers (Birstein 1993). The white sturgeon (*Acipenser transmontanus*) of the Fraser River in British Columbia, Canada, are no exception to this phenomenon, and much has been published in the popular literature in the last 5 years, partially stimulated by two die-offs of very large sturgeon in the summers of 1993 and 1994 (Glavin 1994). Furthermore, their extraordinary longevity (to >100 years of age) and

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large size (>600 kg) (Scott and Crossman 1973) make them unique among North American freshwater fish.

White sturgeon may live out part of their lives in both fresh and marine environments. However, spawning must take place in freshwater (Hanson et al. 1992), and very young white sturgeon are also not tolerant of salt water (McEnroe and Cech 1985; Conte et al. 1988). Nonetheless, as juveniles grow older, their ability to osmoregulate becomes greater, and the frequency of estuarine and marine rearing by older juveniles and adult white sturgeon and their propensity to migrate within and between salt and freshwater appear to be more frequent (Echols 1995). However, these movements are still not well known, particularly for Fraser River populations. The understanding of these movements is important, as conservation and fisheries management efforts are hindered by a lack of information regarding the migratory behaviour of these fish within and outside the Fraser River system.

To better understand the seasonal migratory patterns of the Fraser River white sturgeon and to determine the frequency and duration of any marine migrations, additional data on their movements are needed. Radiotracking studies are expensive and generally only small numbers of white sturgeon are tracked for 2–3 years (e.g., ECL Envirowest Consultants Limited 1992; R.L.&L. Environmental Services Ltd. 1994). Considering that some estimates of white sturgeon age have exceeded 100 years (Rien and Beamesderfer 1994; this study), individuals can only be tracked for a small portion of their lives. Conventional tagging–recapture studies can mark larger numbers of fish, but such studies are labour intensive and recapture rates are generally low (e.g., Rien and Beamesderfer 1994). Also, tagging and radiotracking can only monitor an individual's movements for the duration of the study, and neither gives any information on past movements.

New research techniques are required that can provide information over the entire lives of individuals or populations. Further, the status of the Fraser River white sturgeon populations dictates that any new techniques be nonlethal and as nonintrusive to the white sturgeon as possible. One option is the use of laser ablation sampling – inductively coupled plasma – mass spectrometry (LAS-ICP-MS) (Outridge et al. 1995) to determine the chemical composition of the seasonal growth zones in a section of the leading ray of the pectoral fin of the white sturgeon.

The pectoral fin ray (Fig. 1), a bony structure in the pectoral fin, is a potential historical record of physiological and environmental influences. Under ordinary circumstances the white sturgeon deposits two optically distinct zones of bone on the fin ray each year of its life, and these zones are arranged in a roughly concentric fashion. A section of the fin ray can be removed without sacrificing the fish, and after polishing the section the fish may be aged by counting the number of rings of a given opacity (Rien and Beamesderfer 1994).

Rare elements or isotopes can be incorporated into fish scales, bones, and otoliths (Outridge et al. 1995), and the elemental signature can be used in combination with the age in order to determine the habitat location for a particular life history stage (Kalish 1990; Coutant and Chen 1993; Campana et al. 1994). One element that may be used to track sturgeon movements is Sr. Typical Sr/Ca ratios in the marine environment (0.0087) are significantly higher than in the freshwater environment (0.0019) (Kalish 1990). Strontium is easily substituted for Ca and is deposited in fish scales and otoliths as well as bivalve shells in proportion to the Sr/Ca ratio of the water in which the organism lived (Coutant and Chen 1993; Tzeng and Tsai 1994; Klein et al. 1996). Further, Sr is easily incorporated into bone (Yamada et al. 1987; Koch et al. 1992). Therefore, fin ray material deposited while white sturgeon are in an estuary or at sea would be expected to contain correspondingly higher concentrations of Sr than fin ray material deposited when an individual is living in freshwater.

The purposes of this study were to determine if (*i*) Sr concentrations between early and later fin ray growth of Fraser River white sturgeon were consistently different and great enough to be detected by LAS-ICP-MS, (*ii*) Sr concentrations varied in a manner consistent with the hypothesis that some lower Fraser River white sturgeon congregate in the estuary as subadults, and (*iii*) Sr concentrations in the fin rays showed evidence of regular marine migrations. Landlocked Columbia River white sturgeon were used as study controls with the assumption that the incorporation of Sr in all freshwater environments, whether it be the Fraser River or Columbia River, is similar.

Two null hypotheses were tested. (1) Mean Sr concentrations in the first 15 years of fin ray growth were not greater than the mean Sr concentrations in years 16–30; this tests for estuarine rearing by juvenile fish. (2) Strontium concentrations in the fin rays would not increase fourfold over estimated freshwater Sr concentrations during the adult life history phase; such fluctuations in Sr would suggest marine migrations.

Methods

Thirty-nine fin rays were selected for LAS-ICP-MS. Twenty-nine fin rays came from Fraser River white sturgeon and 10 were from Columbia River white sturgeon (Table 1). White sturgeon greater than 15 years old were classified as adults, and white sturgeon 15 years old or younger were considered subadults. The fin rays were obtained from various fin ray collections. Sturgeons 9701–9713 are from Fraser River white sturgeon captured in the commercial fishery in 1962 (Semakula and Larkin 1968). Sturgeons 9503, 9505, 9654, 9658–9662, and 9714–9718 were collected by the British Columbia Ministry of Environment, Lands and Parks and are from lower Fraser River white sturgeon that washed ashore dead during the summers of 1993 and 1994. Sturgeon 9656 was collected from an angled Fraser River fish caught in 1991. Sturgeons 9626 and 9653 are from Fraser River fish that died in 1977. Sturgeon 9626 was caught live while sturgeon 9653 washed ashore dead. All Fraser River white sturgeon were sampled downstream of Hope, B.C., which is about 150 km from the Strait of Georgia and considered to be the "lower Fraser." No barriers to migration by fish to and from marine environments occur in this reach of the Fraser River. Sturgeons 9668, 9670, 9672, 9674, 9676, 9678, 9680, 9682, 9684, and 9686 are from landlocked Columbia River fish captured live in 1995 and 1996 in either the McNary Reservoir (river kilometres 471–536) or the John Day Reservoir (river kilometres 346–471) (T. Rien, Oregon Department of Fish and Wildlife, Columbia Region, 17330 S.E. Evelyn St., Clackamas, OR 97015-9514, U.S.A., personal communication).

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Fig. 1. Photomicrograph composite of a section of the leading ray from the pectoral fin of a white sturgeon showing examples of opaque (O) and translucent (T) growth zones.

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Table 1. Fin rays used in this study.

Fraser River			Columbia River		
Fin ray	Collection year	Age of white sturgeon (years)	Fin ray	Collection year	Age of white sturgeon (years)
9503	1993	56	9668	1995	12
9505	1994	68	9670	1995	11
9626	1977	132	9672	1995	21
9653	1977	33	9674	1995	20
9654	1994	31	9676	1995	22
9656	1991	33	9678	1995	29
9658	1994	91	9680	1995	23
9659	1994	39	9682	1996	14
9660	1994	73	9684	1996	13
9661	1993	88	9686	1996	17
9662	1994	96			
9701	1962	18			
9702	1962	21			
9703	1962	22			
9704	1962	14			
9705	1962	15			
9706	1962	19			
9707	1962	23			
9708	1962	22			
9709	1962	12			
9710	1962	12			
9711	1962	17			
9712	1962	11			
9713	1962	11			
9714	1994	52			
9715	1993	71			
9716	1994	92			
9717	1993	119			
9718	1993	65			

Table 2. LAS-ICP-MS operating conditions.

Conditions	Value
Laser	
Laser energy (J)	40–45
Repetition rate (Hz)	10
Number of laser shots	50
Q-switch delay (ms)	240–250
ICP-MS	
Forward power (kV)	1050
Plasma gas (L/min)	15.0
Auxillary gas (L/min)	0.80
Nebulizer gas (L/min)	1.0

To prepare fin rays for laser sampling and to enhance distinction of the opaque and translucent zones, fin rays were cross sectioned and polished. Five hundred micrometre thick sections of fin ray were removed using an Isomet low-speed saw with a wafering diamond blade. The fin ray sections were lightly polished by hand with 1200-grit lapidary film until the opaque and translucent bands were clearly visible under a light microscope at 40× magnification. Fin rays were then given a final buffing using 10-μm lapidary film. The final buffing was applied to enhance the appearance of the opaque and translucent zones for photographic work and for selecting ablation sites. Fin ray sections were then rinsed in ethanol and distilled water to remove any surface contamination, air dried, and stored in individual ziplock plastic bags until ablated.

Each white sturgeon was aged by counting the number of opaque zones (annuli) observed in the polished sections of fin rays at 40× magnification. It was assumed that no bands were missing due either to bone resorption or from removing the fin ray too distally from the point of articulation. Each annulus was then assigned a number that corresponded to the age of the white sturgeon at the time of the band production.

Material was extracted from the specimen fin ray using a Perkin Elmer SCIEX laser sampler model 320 operated in the Q-switched mode with the laser beam wavelength set at 0.266 μm. The laser beam was focused onto the specimen through a microscope and could be observed by way of a television monitor. Specimens were held in a closed container, and ablated material was transported to the ICP-MS by a flow of argon. The LAS-ICP-MS was optimized daily to maximize the count rates on ^{88}Sr and ^{44}Ca while maintaining an ablation crater of about 50 μm in diameter. Laser and ICP-MS operating conditions are given in Table 2.

Each fin ray was sampled from its growing edge to its core along the axis of maximum growth. Each annulus in the fin ray was ablated at one site. To minimize the effect of instrument drift on the final results, the direction (edge to core, core to edge) of the sampling traverse was randomized.

Isotopic data were acquired on a SCIEX Elan model 5000 ICP-MS. The ICP-MS provides the user with data in the form of counts per second for each isotope selected for analysis. To account for varying ablation yields between ablation sites, the ratio of ^{88}Sr

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Table 3. Mean Sr values and relative standard deviations (RSD) for multiple laser shots on individual opaque zones in white sturgeon fin rays and on the NIST 1486 pellet.

Fin ray material (ppm Sr)						
Mean	203	183	235	197	229	177
RSD (%)	5	4	3	4	6	4
n	4	4	4	4	4	7
NIST 1486 (Sr/Ca counts)						
Mean	0.08	0.42	0.09			
RSD (%)	5	11	5			
n	5	5	5			

counts to ^{44}Ca counts was calculated (Arrowsmith 1987; Jackson et al. 1992). The ICP-MS was operated in the peak hop mode with the number of replicates set at 50 and a dwell time of 20 ms for a total counting time of 1 s on each isotope. Because of the appearance of apparently random outliers in LAS-ICP-MS data (Outridge et al. 1996), all data were subjected to a variation of the Grubbs test, a test for extreme outliers (Taylor 1990). Mean count rates and standard deviations were calculated for each mass (n = 50) on every ablation site, and outliers were defined as individual count rates that were greater than 3 SD above the mean. Data points deemed outliers were assigned the count rate of the data point immediately preceding them. Backgrounds were calculated from the first two and last 10 (prior to any ablation product reaching the plasma and after signals returned to preablation levels) replicates. Outlier and background-corrected data were converted to concentrations using the equation of Arrowsmith (1987):

$$(1) \qquad \frac{I(\mathrm{E})}{I(\mathrm{IS})} C(\mathrm{IS}) = C(\mathrm{E}) \frac{R(\mathrm{E})}{R(\mathrm{IS})}$$

where I(E) is the count for the element of interest, I(IS) is the count for the internal standard, C(IS) is the concentration of the internal standard, C(E) is the concentration of the element of interest, R(E) is the element response factor for the element of interest, and R(IS) is the element response factor for the internal standard.

The internal standard may be an analyte of known concentration, a matrix element, or an added element (Arrowsmith 1987). We used ^{44}Ca, a matrix element, as the internal standard. The mean total Ca concentration in a sample of fin rays was determined by atomic absorption spectrometry and set at 26% by weight. The R(E)/R(IS) or relative sensitivity factor was determined for ^{88}Sr based on laser analyses of a pellet made from National Institute of Standards and Testing (NIST) standard reference material (SRM) No. 1486, a bone meal. Strontium concentrations calculated using eq. 1 are in essence Sr/Ca ratios normalized to the Sr/Ca ratio in the standard. However, concentrations are reported as parts per million rather than ratios to make comparisons between fin rays easier. As well, the mass superscripts are no longer necessary and will not be used in further discussions.

To monitor instrument drift within and between days of analyses, a fin ray (Trent-1) was selected as an in-house standard. Trent-1 was analyzed at a minimum of eight ablation sites per day. Strontium concentrations in Trent-1 were compared by an analyses of variance to determine if day of analyses was a significant factor in determining Sr concentrations in Trent-1.

To determine the precision of the technique, means and standard deviations were calculated from multiple laser shots on the NIST 1486 pressed pellets and within individual annuli on six additional fin rays that were not analyzed for any other part of this study.

In addition to testing for spurious data points due to the ablation process, a Grubbs test (Taylor 1990) was also used to determine if any Sr concentrations in the fin rays could be classified as outliers. Individuals in which a Sr outlier was detected were assumed to have spent part of their life in an estuarine or marine environment. Individuals in which a Sr outlier concentration was ≥400 ppm were assumed to have been in a marine environment.

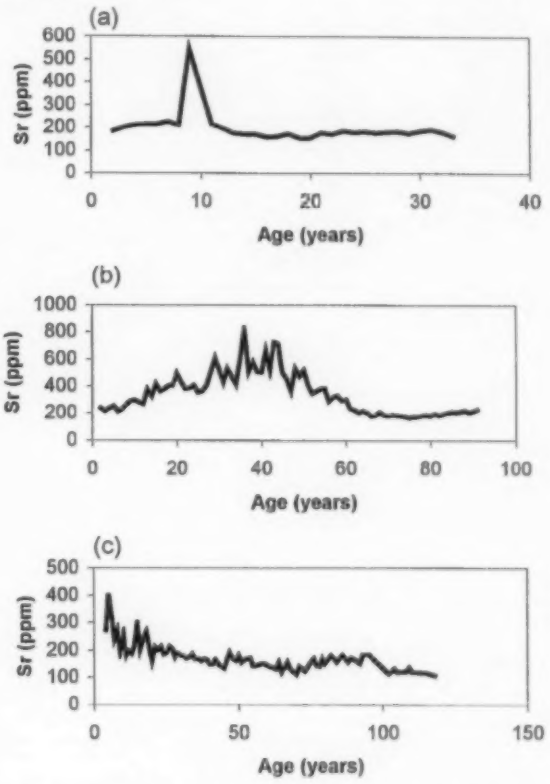

Fig. 2. Relationship between age of Fraser River white sturgeon at the time of production of an annulus and the Sr concentration in the annulus. Each individual is believed to have made at least one marine migration. (*a*) Sturgeon 9653; (*b*) sturgeon 9658; (*c*) sturgeon 9717.

To test for exposure of juvenile white sturgeon to a Sr/Ca environment significantly different from the Sr/Ca environment in which mature individuals lived, t tests were performed on individuals greater than 15 years old. Mean Sr concentrations in fin ray material produced in the first 15 years of life were compared with the mean fin ray Sr concentrations in fin ray material from years 16 to 30 or in the remainder of the fin ray if an individual was less than 30 years old. Individuals with fin ray Sr concentrations in the first 15 years of fin ray growth significantly greater than the mean fin ray Sr concentration in years 16–30 were also classified as having spent time in either an estuarine or a marine environment.

Results

Precision

Mean Sr concentrations and relative standard deviations for multiple laser shots on NIST 1486 and individual annuli of six additional fin rays (not used in other parts of this study) are given in Table 3. Within growth zone relative standard deviations were less than 8% for all annuli, and relative standard deviations were less than 12% for the SRM.

There were significant differences in the mean Sr concentrations in Trent-1 between days ($p < 0.05$), but the range of

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Table 4. Strontium concentrations (ppm) determined in fin rays from adult white sturgeon.

Sturgeon	Sr years 1–15 (minimum–maximum)	Sr years 16–30 (minimum–maximum)	p	Outlier
Fraser River				
9503	147 (126–196)	132 (108–149)	<0.01	Yes
9505	194 (174–212)	221 (198–245)	<0.01	No
9626	154 (145–172)	148 (143–161)	0.03	Yes
9653*	224 (170–545)	171 (152–184)	0.04	Yes
9654	206 (196–216)	199 (183–243)	0.1	No
9656	178 (149–199)	181 (153–200)	0.3	No
9658*	227 (211–414)	410 (350–603)	<0.01	Yes
9659	239(183–298)	241 (174–317)	0.4	No
9660	188 (160–209)	148 (137–157)	<0.01	No
9661	193 (155–236)	140 (121–167)	<0.01	Yes
9662	261 (192–347)	152 (135–193)	<0.01	Yes
9701	211 (185–264)	254 (220–273)	<0.01	No
9702	219 (194–245)	193 (176–221)	<0.01	No
9703	248 (196–344)	204 (176–238)	0.03	No
9706	215 (190–245)	255 (214–298)	<0.01	Yes
9707	228 (199–270)	197 (179–227)	<0.01	No
9708	224 (188–250)	188 (174–202)	<0.01	No
9714	176 (146–230)	146 (127–160)	<0.01	Yes
9715	227 (193–277)	249 (192–311)	0.06	No
9716	238 (221–285)	210 (187–241)	<0.01	No
9717*	251 (177–406)	201 (161–264)	<0.01	Yes
9718	165 (146–192)	177 (147–203)	0.04	No
Columbia River				
9672	190 (166–229)	191 (176–207	0.5	Yes
9674	213 (155–279)	231 (189–259)	0.2	No
9676	260 (226–299)	211 (187–262)	<0.01	No
9678	233 (203–280)	253 (221–273)	0.08	No
9680	236 (181–263)	168 (157–183)	<0.01	No
9686	218 (182–256)	225 (203–147)	0.3	No

Note: Values given are mean Sr concentrations for annuli produced in years 1–15 and years 16–30 followed by the minimum and maximum Sr concentrations (in parentheses) determined over the same growth periods. The probability of a difference in mean values between years 1–15 and years 16–30 is given as the p value. The presence of a Sr outlier is indicated by a "yes" response in the outlier column. An asterisk indicates a fish believed to have made at least one marine migration.

the mean Sr concentrations in Trent-1 for the entire study period was only 38 ppm. Therefore, it was decided not to correct Sr concentrations for day of analysis.

Marine Sr signatures

Three white sturgeon only produced fin ray material with Sr concentrations greater than our marine Sr signature minimum value of 400 ppm (Fig. 2). All three fish were captured in the Fraser River, which is about 10% of our Fraser River sample. In each case the highest Sr concentration in each individual was classified as an outlier (Grubbs test, $p < 0.05$). It was assumed, then, that these three fish spent some part of their lives in a marine environment.

Fin ray Sr concentrations in early and later growth

There was no significant difference in the mean fin ray Sr concentration of the nonmarine Fraser River fish and the Columbia River fish (t test, $p < 0.05$, df = 37). However, there were differences in the general distribution of Sr within the fin rays of white sturgeon from the two rivers. Eleven of the 19 (58%) nonmarine adult Fraser River fish had mean Sr concentrations in the first 15 annuli of their fin rays significantly greater (t test, $p < 0.05$) than the mean Sr concentrations in the annuli produced from age 16 to 30, four of 19 (21%) had significantly lower mean Sr concentrations in the first 15 annuli compared with annuli 16–30, and four of 19 (21%) showed no difference in the Sr concentrations between early and later growth (Table 4). In the Columbia River sample, two of the six (33%) adult fish had significantly higher mean Sr concentrations in the first 15 annuli of their fin rays compared with the later growth in the fin ray, while the remaining fin rays (66%) had no significant differences in Sr concentrations in the early and later growth (Table 4). Furthermore, 10 of the Fraser River fish had Sr concentrations in their fin rays classified as outliers (Grubbs test, $p < 0.05$) (Tables 4 and 5), whereas only one Columbia River fish produced a Sr outlier (Table 4). Assuming that outliers are caused by a significant change in the Sr/Ca ratio of their environment, the data suggest that these Fraser River white sturgeon were exposed to a more variable water chemistry than were the landlocked Columbia River fish.

Strontium data in the fin rays from subadult fish from the two rivers were also dissimilar. A Sr outlier was detected in one Fraser River subadult fish, and a second individual (stur-

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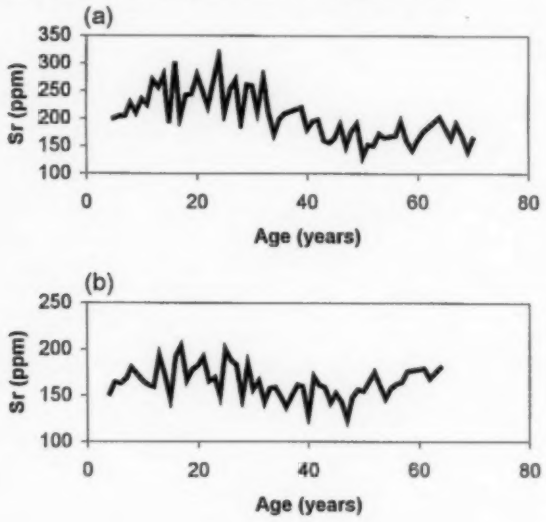
Table 5. Strontium concentrations (ppm) determined in fin rays from subadult white sturgeon.

Fraser River			Columbia River		
Sturgeon	Sr (ppm)	Outlier	Sturgeon	Sr (ppm)	Outlier
9704	232 (197–289)	Yes	9668	195 (177–224)	No
9705	224 (171–248)	No	9670	188 (165–220)	No
9709	247 (228–270)	No	9682	235 (211–270)	No
9710	263 (211–297)	No	9684	252 (219–282)	No
9711	182 (162–202)	No			
9712	230 (219–248)	No			
9713	286 (214–374)	No			

Note: Values given are mean Sr concentrations followed by the minimum and maximum Sr concentrations (in parentheses). The presence of a Sr outlier is indicated by a "yes" response in the outlier column.

Fig. 3. Relationship between age of Fraser River white sturgeon at the time of production of an annulus and the Sr concentration in the annulus. (*a*) Sturgeon 9505; (*b*) sturgeon 9718.



geon 9713) produced a Sr concentration of 374 ppm, which is very close to our 400-ppm minimum marine value (Table 5). The Columbia River subadults produced no Sr outliers, and the maximum Sr value produced was 282 ppm (Table 5).

Discussion

Precision and accuracy of LAS-ICP-MS

LAS-ICP-MS has not been widely used as a quantitative analytical technique due largely to the poor precision and accuracy of the technique. However, others have reported precise and accurate results for certain elements including Sr (Denoyer et al. 1991; Williams and Jarvis 1993; Raith et al. 1996). The precision shown in Table 3 for the fin ray material support these findings, but Table 3 also shows that the Sr/Ca ratios measured on a SRM can change significantly among analyses days (0.08–0.42). This is believed to be caused by changes in the ablation characteristics of the laser beam. Therefore, to compare data among days, it was necessary to normalize the Sr/Ca ratios measured on the fin rays to an SRM (eq. 1). As an additional check on the day-to-day variability of the data, an in-house standard should also be analyzed.

The precision obtained in this study and the small changes in the absolute concentration obtained on our in-house standard (total range of 33 ppm) should allow us to detect large changes in Sr concentrations within fin rays and to detect changes in mean Sr concentrations in the earlier and later growth regions of the fin rays.

Evidence of marine migrations

The observed changes in the Sr concentrations of our fin rays cannot be attributed to any specific cause, since none of these fish have a known exposure history. Changes in Sr values could be caused by changes in the water chemistry of the river, changes in diet throughout the life of the individual fish, or migration to an estuarine or marine environment. However, many studies have shown that migrations to estuarine or marine environments will produce two- to three-fold changes in the Sr concentrations of bone, scales, and otoliths of many different species (Kalish 1990; Koch et al. 1992; Coutant and Chen 1993; Tzeng and Tsai 1994). Since (*i*) white sturgeon have been sighted, captured, and others found dead in marine environments, (*ii*) seasonal changes in Fraser River water chemistry are minor (Cameron 1996), and (*iii*) there is little migration of Sr among the annuli of white sturgeon fin rays (Veinott and Evans 1999), it seems reasonable to assume that a marine migration during the spring or summer growth period by any of our fish would produce large and distinctive changes in the Sr concentrations of their annuli.

Kalish (1990) showed that there was an approximate fourfold difference in the Sr/Ca ratios between fresh and marine waters. Thus, bone, scales, or otoliths produced in equilibrium with the water would be expected to show similar differences in Sr/Ca ratios. Indeed, the otoliths in the Kalish (1990) study, which were exposed to a marine environment, did exhibit Sr/Ca ratios that were distinct and about at ratios similar to those found in seawater (threefold). Nonetheless, we could not be sure that fin rays would incorporate Sr and Ca in the same proportions as otoliths. Therefore, we decided that, as a starting point, we would look for fin rays with a fourfold increase in Sr concentrations (Sr/Ca ratios normalized to an SRM (eq. 1)) and identify any such fin rays as having come from fish that had made at least one marine migration. The baseline against which the fourfold increase was measured was the 100- to 200-ppm Sr values of older (40+ years) annuli that showed little variation in the Sr con-

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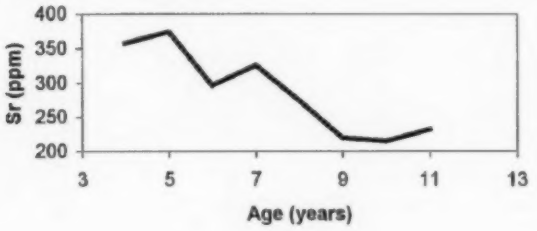
Fig. 4. Relationship between age of Fraser River white sturgeon (sturgeon 9713) at the time of production of an annulus and the Sr concentration in the annulus.



centrations (Figs. 2*c* and 3). It was assumed that these values represented the Sr concentrations of individuals that had taken up permanent residence in the river.

Only three individuals (all Fraser River fish) from this study produced fin ray material with Sr concentrations in the marine range (400–800 ppm) (Fig. 2). In each case the highest value was identified as an outlier by the Grubbs test (Taylor 1990), which detects sudden changes in a series of values. This suggests that white sturgeon in the lower Fraser River do not make seasonal migrations to the sea.

Our conclusion that marine migrations by Fraser River white sturgeon are not periodic is not based on a large sample of fin rays from the lower Fraser River white sturgeon population. We suggest that this study is the first stage in developing a new technique for examining fin ray chemistry in an attempt to better understand the life history of the white sturgeon. Whether our 29 Fraser River white sturgeon are representative of the total population can only be addressed by further sampling. Nevertheless, there were no physical barriers preventing any of the Fraser River fish from migrating to the sea, and our data agree with a more extensive tagging and recapture study of the lower Columbia River white sturgeon population (DeVore and Grimes 1993) that suggested that marine migrations were rare among individuals and highly variable in duration. Therefore, our results are consistent with the hypothesis that migrating to a marine environment is not a common life history behaviour for white sturgeon.

Evidence of time spent in an estuary

Subadult Fraser River white sturgeon are regularly sighted and captured in the Fraser River estuary (Echols 1995). Likewise, DeVore and Grimes (1993) concluded that in the Columbia River, subadult white sturgeon congregated in the estuary during the summer. Since an estuary is a mix of fresh and marine waters, bone produced in this environment would be enriched in Sr, compared with freshwater, by some intermediate factor less than 4 used to indicate exposure to a marine environment.

Within the nonmarine adult Fraser River white sturgeon sample, 58% of the individuals had Sr concentrations in their first 15 annuli (when they were subadults) significantly higher ($p < 0.05$) than the Sr concentrations in the annuli produced during years 16–30, and five of these individuals had Sr concentrations identified as outliers (Table 4). These outliers were not within the 400- to 800-ppm range expected of fin ray material produced in a marine environment, but they were as much as 1.5- to 2-fold higher than the 100- to 200-ppm base level. It was assumed, therefore, that these individuals spent their early years either in an estuarine environment or migrating to the estuary periodically. Other nonmarine adult white sturgeon that did not meet the criteria for estuarine migrations, but may have spent time in the estuary, include individuals with Sr outliers, or those with Sr concentrations exceeding 300 ppm.

The data from sturgeon 9713, an 11-year-old fish (Fig. 4), also show that high Sr concentrations in the earlier annuli are not restricted to older individuals. In contrast, sturgeons 9715 and 9718 exceed 60 years of age (Fig. 3) but exhibit no increase in Sr in the early growth zones of their fin rays. Increased Sr concentrations in the early growth zones of the fin rays do not appear to be age related and are more likely caused by a change in the Sr/Ca environment in which those fin rays grew. Fish in this age-class (subadults) are probably selectively foraging in environments that are rich in food, and the Fraser River estuary affords this opportunity for quick growth.

Columbia River Sr signatures

Since the Fraser River white sturgeon in this study were not of a known life history, fin rays from "landlocked" Columbia River reservoir fish were used as controls. All the white sturgeon used in this part of the study were captured in the same reservoirs 2–6 years previous. To reach the reservoirs from the sea, these fish would have had to pass through a minimum of three fishways and travel a minimum of 346 km from the estuary. White sturgeon in these reservoirs normally do not make mass migrations through the fishways, and only a maximum of 30 a year have been observed moving between reservoirs (Warren and Beckman 1993). The Columbia River white sturgeon used in this survey were, therefore, assumed not to have made seasonal migrations to the sea or the estuary.

However, mean Sr concentrations in the first 15 years of fin ray growth for two Columbia River individuals were significantly higher than the mean Sr concentration for the remainder of the growth of the fin ray (Table 4), and a third individual produced a Sr outlier at age 12 (sturgeon 9672) (Table 4). Therefore, either our assumption of nonmigration by these individuals is incorrect or comparing the Sr concentration in early and later fin ray growth and identifying Sr outliers is not an accurate method for identifying estuarine or marine fish. Without fish of a known migratory history, it is not possible to reject either of these possibilities. However, the range and maximum Sr values reported for the Columbia River individuals are much less than those for the Fraser River individuals identified as having made marine migrations. Further, all Sr concentrations for Columbia River fish are above 150 ppm (Tables 4 and 5), suggesting that the baseline Sr values for the Columbia River fish may be higher than for the Fraser River fish, possibly due to slight differences in the water chemistry of the two rivers.

Implications for white sturgeon management and habitat protection

Understanding the habits and movements of white sturgeon is beneficial to managing the stock. Known habitats can be monitored and protected, particularly during times of

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high use, and incidental catch rates may be reduced through white sturgeon awareness programs. Clearly, additional information is needed to fully understand and conserve this stock.

Our data suggest that white sturgeon in the lower Fraser River rarely migrate to sea. Furthermore, after about 40 years of age, most Fraser River white sturgeon are permanent residents of the river and make no migrations to the sea or the estuary. This is consistent with radiotracking studies that repeatedly located tagged white sturgeon in the same area (ECL Envirowest Consultants Limited 1992; R.L.&L. Environmental Services Ltd. 1994) or found no evidence of marine migrations (R.L.&L. Environmental Services Ltd. 1994). A lack of population mixing would put these discrete components of the population, including reproducing fish, in a vulnerable position with respect to fishing pressure or habitat destruction.

Acknowledgments

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Population differences in antifreeze production cycles of juvenile Atlantic cod (*Gadus morhua*) reflect adaptations to overwintering environment

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Abstract: Four groups of juvenile Atlantic cod (*Gadus morhua*) were collected in summer (1992) from four coastal sites of northeast Newfoundland. They were held together at seasonally ambient temperature and photoperiod and monitored over one winter for variation in a biochemical phenotype, antifreeze production capacity. Our results show that population differences in antifreeze production capacity reflect the severity of overwintering conditions faced. Cod from three bays along the northeast coast (49°30′–47°30′ N) exhibited very similar patterns of antifreeze production, suggesting that these environments demand from their cod inhabitants approximately the same high level of freeze resistance. However, cod from the most exposed northerly site at the tip of the Great Northern Peninsula (51°30′ N) developed antifreeze levels about 50% higher than those of the other three groups, confirming that the more extreme high-latitude environment demands even greater freeze protection for survival. Since 34% of Great Northern Peninsula cod produced antifreeze levels outside the range of all other juveniles, we suggest that the physiological distinctness of this population has evolved by genetic amplification of antifreeze production capacity rather than by selective mortality. The existence of physiological population structure should be factored into strategies for sustainable fishing of this species in northern waters.

Résumé : Des morues de l'Atlantique (*Gadus morhua*) juvéniles ont été prélevées au cours de l'été (1992) dans quatre sites côtiers du nord-est de Terre-Neuve. Les morues ont été conservées ensemble dans des conditions de température et de photopériode naturelles et ont fait l'objet d'un contrôle de la variation d'un phénotype biochimique - la capacité de production d'une molécule antigel (CPA) pendant un hiver. Les résultats obtenus montrent que les écarts entre populations de la CPA traduisent la sévérité des conditions d'hivernage. Les morues provenant de trois baies de la côte nord-est (lat. de 49°30′–47°30′ N) présentaient des allures de production de molécules d'antigel très semblables, ce qui porte à croire que ces environnements exigent des morues une résistance élevée au gel qui est similaire. Les morues provenant de la zone la plus exposée, du site situé au nord, à l'extrémité de la péninsule Great Northern (lat. 51°30′ N), ont présenté des teneurs de molécules d'antigel de 50% environ supérieures à celles des morues des trois autres groupes, ce qui confirme que l'environnement de latitude plus élevée exigeait une plus grande protection contre le gel pour assurer leur survie. Étant donné que 34% des morues de la péninsule présentaient des teneurs d'antigel débordant de la gamme de celle de toutes les autres morues juvéniles, nous proposons que cette caractéristique physiologique de la population s'explique par une amplification génétique plutôt que par une mortalité sélective. L'existence de la structure physiologique des populations devrait être prise en compte dans les stratégies de pêche durable de cette espèce dans les eaux du nord.

[Traduit par la Rédaction]

Introduction

At high latitudes, ocean temperatures frequently fall well below 0°C and can reach the freezing point of seawater (about –1.8°C); ice cover can also be extensive (Prinsenberg et al. 1997). Such habitats are lethal to most species of teleost fish because their body fluids generally freeze at between –0.5 and –0.8°C if they come into contact with ice (Holmes and Donaldson 1969). The fish species that inhabit such environments have developed a mechanism for surviving these extreme conditions: they produce plasma antifreeze proteins or glycoproteins (AF(G)Ps) that improve their freeze resistance. These AF(G)Ps have a particular affinity with the ice crystal lattice and bind to embryonic ice crystals, preventing their further growth. In this manner, the AF(G)Ps are able to lower the freezing point of the blood and extracellular fluids below that attributable to the colligative properties of the solutes contained therein (DeVries 1983; Kao et al. 1986; Davies et al. 1988; Cheng and DeVries 1991).

To date, five distinct classes of AF(G)Ps have been described in a wide variety of fish taxa. These proteins, their genes, and their functional significance to the survival of fish

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in icy marine waters appear to have developed in response to a major environmental change between 30 million and 2.5 million years ago that resulted in sea level glaciation (Davies et al. 1993; Cheng 1998). Prompted by the selective forces of a changing environment, AF(G)P genes are believed to have evolved from preexisting gene sequences already encoding for proteins with ice-binding potential. Gene amplification most likely provided the method by which a rapid increase in the amount of gene product (AF(G)P) could be achieved (Davies et al. 1993). In the face of falling environmental temperatures, such gene amplification events, resulting in increased antifreeze production and cold tolerance, would confer considerable selective advantage to those fish in which they occurred. The most extreme environments would exert the greatest selection pressure, and thus, one might expect to see environmental and physiological coupling in terms of winter severity and levels of AF(G)P production.

Atlantic cod (*Gadus morhua*) produce antifreeze glycoproteins (AFGPs) in response to low environmental temperatures (Fletcher et al. 1987; Goddard et al. 1992). Previous research on adult cod (>40 cm in length) collected mainly from Conception Bay, Newfoundland (NAFO Division 3L), showed that although they produced AFGPs during the winter, which significantly improved their freeze resistance over that conferred by the plasma electrolytes, the adults studied did not produce sufficient antifreeze to protect them from freezing if the water temperatures declined below about −1.2°C (Goddard and Fletcher 1994). In contrast, juvenile cod (15–40 cm) collected from the same general area as the adults produced about twice as much AFGP as did the adults, resulting in a level of freeze resistance approaching −1.5°C (Goddard and Fletcher 1994). These differences in AFGP levels between adult and juvenile cod are thought to be related to what is generally known about their differences in overwintering behaviour: the adults migrate to warmer (>0°C) offshore locations, or remain inshore in cold water but stay deep to avoid surface ice, while juveniles are reported in inshore areas characterized by low temperatures and ice where higher antifreeze levels would be advantageous (Goddard et al. 1992; Goddard and Fletcher 1994). Thus, the differences in maximum winter antifreeze levels between adult and juvenile cod can be seen as an ontogenetic physiological adaptation to the overwintering environment occupied.

This study was designed to test the hypothesis that AFGP production capacity in Atlantic cod is coupled with the environment inhabited and that cod populations inhabiting the most extreme winter environments will have evolved the greatest antifreeze production capacity. The cod used were taken from the northern cod stock (cod inhabiting NAFO Divisions 2J, 3K, and 3L off the east coast of Newfoundland and Labrador). With increasing latitude, the environment inhabited by northern cod becomes progressively more severe, in terms of prevalence of low water temperatures and the extent and persistence of ice cover in winter and spring, a situation that might be expected to result in population differences in AFGP production.

Northern cod have, in the past, been managed as a single stock complex. There is now considerable evidence that northern cod are not panmictic, and studies of tagging, morphometric, meristic, and genetic data suggest that several discrete populations may exist within the 2J3KL management unit (Myers et al. 1997). We wished to elucidate whether a physiological characteristic (AFGP production capacity) could be added to the list of indicators of population discreteness.

Before looking for population differences, it is relevant to know the initial geographic origin of the experimental animals. This presents a problem when studying Atlantic cod in the Newfoundland region because they tend to be highly mobile as adults, carrying out extensive migrations during the course of a year (Templeman 1979; Rose 1993; Taggart et al. 1995). Thus, location of collection alone would not necessarily give a true indication of stock origin in adult cod. While information on the extent of movements of juvenile cod is very limited, there is evidence to suggest that immature cod developing inshore in Newfoundland waters do not join the migrating adults until they become late juveniles/young adults at about age 4 and length 40+ cm (Rose 1993) and that they maintain a level of site fidelity to their inshore nursery grounds up to age 3 (Clark and Green 1990). Current models of egg and larval drift for northern cod suggest that the juvenile cod population of any bay may be the result of inshore spawning in that area or of a spawning event further north, since the major near-surface currents off the coast of Labrador and northeast Newfoundland flow from north to south (Anderson and Dalley 1997).

Thus, the following investigation was carried out on pre-migratory juveniles, based on the assumption that juveniles from each collection site were the result of spawning at that general location or of a spawning event further north. Four groups of cod, collected from four different geographical areas along the northeast coast of Newfoundland, were compared for their capacity to produce antifreeze over a winter period and thus withstand environmental conditions of low temperature and ice.

Materials and methods

During the summer of 1992, juvenile Atlantic cod were collected from four sites on the northeast Newfoundland coast: Conception Bay (Holyrood), Trinity Bay (Bellevue), Notre Dame Bay (Brighton), and the Great Northern Peninsula (St. Lunaire) (Fig. 1). Cod were collected either from cod traps (Department of Fisheries and Oceans, Northern Cod Science Program) or by beach seining and were transported to the Ocean Sciences Centre, Memorial University of Newfoundland. By October 1992, all experimental animals had been collected and delivered to the Ocean Sciences Centre.

On arrival, all cod were tagged using passive integrated transponder (PIT) tags (BioSonics) to allow identification of each individual fish. One cylindrical glass-coated PIT tag (approximate external dimensions 11 mm long × 2 mm in diameter) was inserted into the peritoneal cavity of each experimental animal. For this process, cod were anaesthetized in a 0.06 $g \cdot L^{-1}$ solution of tricane methanesulfonate (MS 222, Sandoz) in seawater. Once unconscious, cod were removed from the anaesthetic, a small slit (about 4 mm long) was made along the ventral midline, and the PIT tag was slipped into the peritoneal cavity. No stitches were required, and in all cases, the slit had completely healed by the first sampling date (November 30, 1992). Once tagged, cod from all four groups were put together into the same tank (2.44 × 1.22 m, water depth 0.9 m) supplied with aerated running seawater at seasonally ambient temperature and exposed to seasonally ambient photo-

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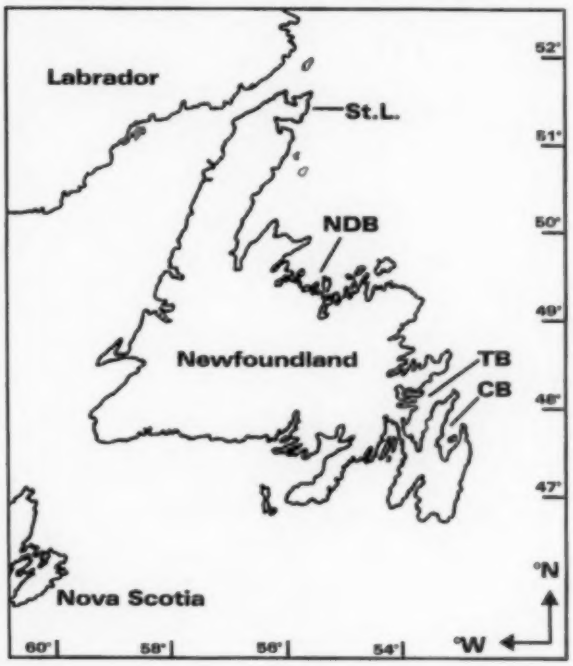
Fig. 1. Location of collection sites of the juvenile Atlantic cod used in all experiments. St.L., St. Lunaire (tip of the Great Northern Peninsula); NDB, Notre Dame Bay (Brighton); TB, Trinity Bay (Bellevue); CB, Conception Bay (Holyrood).



period. Cod from the four locations were all held in this tank until September 1993, at which point the experiment was concluded.

Blood samples were taken from the cod between November 1992 and June 1993 at about monthly intervals using 1-cm^3 syringes fitted with 25-gauge needles (see below for sampling schedule). Prior to taking a blood sample, cod were very lightly anaesthetized in a 0.06 $g \cdot L^{-1}$ solution of MS 222. Once the effect of the anaesthetic could be seen, cod were removed from the water, PIT tag numbers were read electronically and recorded for individual identification, the length of each fish was recorded, and about 0.5 mL of blood was taken from a caudal blood vessel. Each blood sample was placed immediately into a Vacutainer containing sodium heparin (Becton Dickinson) on ice and subsequently centrifuged for 10 min at 4000 × *g*, after which the plasma was removed from the red cells. Plasma samples were stored frozen at –20°C prior to analysis, which was carried out blind and at random. Once blood had been taken, all cod were placed in the same recovery tank (also at ambient temperature and photoperiod) until blood samples had been taken from all experimental animals, at which point, they were all returned to their holding tank. Blood samples were taken on November 30, 1992, and January 8, February 23, April 3, May 12, June 28, and September 21, 1993. In some months, the sampling procedure took 2 days due to the numbers of blood samples being taken. In each of these cases, the date on which the majority of samples were taken has been used in subsequent analyses.

The freeze-depressing action of the AF(G)Ps is due to their particular affinity for, and interaction with, developing ice crystals and is about 200–300 times greater than would be predicted based on the number of antifreeze molecules in solution (i.e., their colligative properties) alone (DeVries 1971, 1988). However, they exert only the normal colligative effects on ice crystal melting points, and thus, a discrepancy or difference exists between the freezing and melting points of a solution containing antifreeze. This difference is termed thermal hysteresis (TH) and is directly proportional to the concentration of antifreeze present in solution (Kao et al. 1986). The technique used here for determining antifreeze activity in small plasma samples makes use of this phenomenon.

Freezing and melting points, and hence, TH values, were determined for each plasma sample using the method described by Kao et al. (1986). With this method, the melting and freezing behaviour of a single ice crystal grown within the test solution is observed microscopically as the sample temperature is raised and lowered using a nanolitre osmometer (Clifton Technical Physics, Hartford, N.Y.). In this way, the sample melting and freezing points can be determined. One is subtracted from the other to give the TH value, and hence, a measure of antifreeze activity within each plasma sample. Past studies using this technique have shown that TH values of between 0.05 and 0.08°C are generally recorded for teleost plasma in summer, when no antifreeze is present (Fletcher et al. 1985*a*, 1987). Such values give an indication of the sensitivity of the method rather than the presence of antifreeze, since blank solutions give similar TH values (generally 0.06°C). TH data may be presented as recorded by the osmometer, when values up to 0.08°C should be interpreted to mean that no antifreeze is present (Fletcher et al. 1987; Goddard et al. 1992). Alternatively, blank values can be subtracted from the measured TH values, giving values approximating 0°C TH in the absence of antifreeze. In this paper, we have used the first option, and thus, in the following results, values less than 0.08°C are indicative of no antifreeze in the plasma.

Cod were fed to satiation with chopped capelin at least twice a week during the cold months and three times a week as the water temperatures increased. Throughout the course of the experiment, water temperatures in the experimental tank were taken about five times a week using a "Quick" thermometer (Cole-Palmer model 8517-00) accurate to 0.1°C.

At each sampling date, the levels of TH, and hence, antifreeze produced by each group, were compared between groups using one-way analysis of variance and Duncan's multiple-range test (Freund et al. 1986).

Results

Juvenile Atlantic cod from all four locations were examined for antifreeze activity at the start of the winter season (November 30, 1992), at which time, no activity could be detected in the plasma of any of the fish (Fig. 2). By January 8, 1993, all four groups of juveniles were producing AFGPs. However, this sampling revealed a divergence between the antifreeze levels produced by the group of juveniles caught at St. Lunaire at the tip of the Great Northern Peninsula and those of the three groups caught in bays on the northeast coast of Newfoundland. The mean antifreeze level of the St. Lunaire juveniles was significantly higher ($p < 0.001$) than those of all the other groups, and this was maintained throughout the winter (Fig. 2). The level of freeze protection developed by the St. Lunaire juveniles would have rendered the majority of them resistant to freezing under almost any circumstances that they might encounter in the ocean.

The difference between the juveniles collected from St. Lunaire and all the other groups reached maximum by April (Fig. 2). Of the St. Lunaire juveniles, 15 fish out of a total of 32 had TH levels >0.9°C, while 11 of these fish had TH levels >1.0°C, a level not seen in any of the other groups. Figure 3 shows the distribution of TH values throughout

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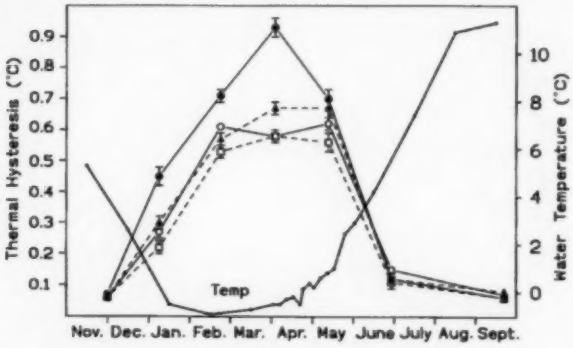
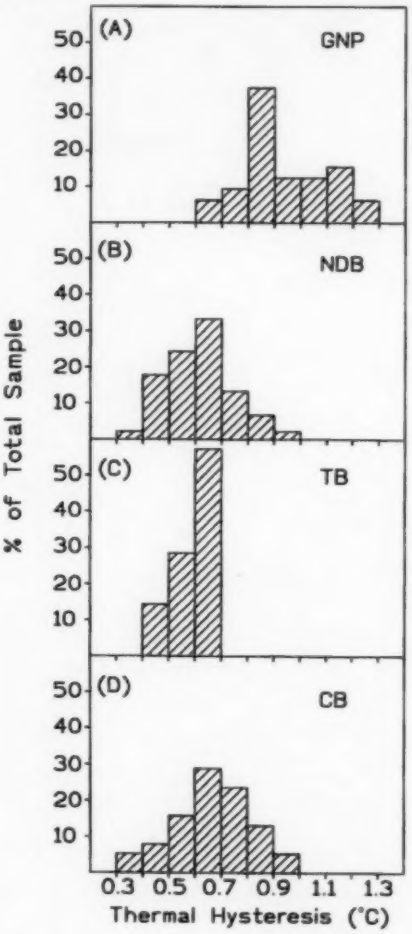
Fig. 2. Patterns of antifreeze production in juvenile Atlantic cod from four locations along the northeast Newfoundland coast: Conception Bay (triangles), Trinity Bay (squares), Notre Dame Bay (open circles), and St. Lunaire at the tip of the Great Northern Peninsula (solid circles). Cod were held at ambient temperature and photoperiod over a winter cycle and sampled for plasma antifreeze activity (measured as TH) between November 30, 1992, and September 21, 1993. All values of TH are presented on the graph as means ± 1 SE. Water temperatures are monthly means plotted at the midpoint of each month with the exception of April–May, for which individual temperature values are plotted every 5 days approximately. Statistical comparison of TH between groups was carried out by one-way analysis of variance followed by Duncan's multiple-range test. Groups assigned a different Duncan's grouping, as indicated by A, B, or C, differed significantly ($p < 0.001$). Groups assigned the same letter were not significantly different. Differences between groups were calculated at the 0.05 level. Groupings from Duncan's multiple-range test for each sampling where there were significant differences between groups and total numbers of fish sampled per month (in parentheses) were as follows. January 8, 1993: Conception Bay = B (32), Trinity Bay = B (24), Notre Dame Bay = B (32), St. Lunaire (Great Northern Peninsula) = A (30); February 23, 1993: Conception Bay = BC (41), Trinity Bay = C (22), Notre Dame Bay = B (49), St. Lunaire (Great Northern Peninsula) = A (33); April 3, 1993: Conception Bay = B (38), Trinity Bay = C (21), Notre Dame Bay = C (48), St. Lunaire (Great Northern Peninsula) = A (32); May 12, 1993: Conception Bay = AB (35), Trinity Bay = C (21), Notre Dame Bay = BC (45), St. Lunaire (Great Northern Peninsula) = A (31).



each group at the time of maximum TH levels. The measured values of TH for the Notre Dame Bay juveniles took a slight dip in April and were not representative of the maximum level (Fig. 2). Thus, in Fig. 3, we have used the May Notre Dame Bay values for comparison with the other three groups (levels in the Conception and Trinity Bay groups were relatively stable between April and May).

In November and January, only a representative sample from each group was examined for antifreeze activity. In February, every fish in each group was sampled; the lengths (centimetres) (mean ± 1 SE, range (number sampled)) in each group were as follows: Conception Bay, 26.7 ± 0.58, 19.5–33.5 (41); Trinity Bay, 31.7 ± 0.52, 27.5–37.0 (22); Notre Dame Bay, 30.9 ± 0.42, 24.4–38.0 (49); St. Lunaire, 28.2 ± 0.53, 23.5–37.0 (33). A previous study has shown that there may be a correlation between length and antifreeze levels in juvenile (age 0+ to 3+) Conception Bay cod (Goddard et al. 1992). We looked for a length effect at the

Fig. 3. Frequency distribution of Atlantic cod TH values attained at the sampling date of maximum antifreeze levels during the winter of 1992–1993. (A) Great Northern Peninsula (GNP), sampling date April 3, $N = 32$; (B) Notre Dame Bay (NDB), sampling date May 12, $N = 45$; (C) Trinity Bay (TB), sampling date April 3, $N = 21$; (D) Conception Bay (CB), sampling date April 3, $N = 38$.



time of peak antifreeze levels (April) by plotting TH against length for each individual fish (Fig. 4) and carrying out a linear regression analysis for each group. The slope of best fit was not significantly different from zero for any of the four groups; no effect of length on antifreeze levels was detected.

Antifreeze levels developed by juveniles from Notre Dame, Trinity Bay, and Conception Bay all presented a similar pattern throughout the winter, and any statistical differences between these three groups that were detected on a monthly basis were not consistent over time (Fig. 2). However, Fig. 3 reveals that the distribution of maximum TH values is very much tighter in the Trinity Bay juveniles than in any of the other groups. The Conception Bay, Notre Dame Bay, and St. Lunaire groups had TH values spread out over a

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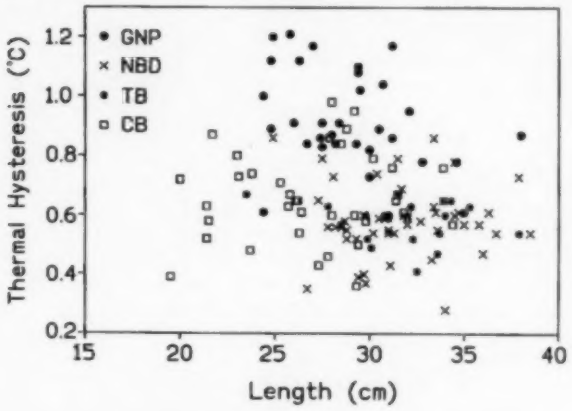
Fig. 4. Plasma antifreeze activity measured as TH plotted against length for each juvenile Atlantic cod sampled on April 3, 1993. All cod from all four collection sites along the northeast Newfoundland coast are represented. Collection sites were St. Lunaire, Great Northern Peninsula (GNP), Notre Dame Bay (NDB), Trinity Bay (TB), and Conception Bay (CB).



0.7°C range, while TH levels of the juveniles from Trinity Bay were all within a 0.3°C range.

The St. Lunaire juveniles started to lose their antifreeze in advance of the other three groups once the winter was over (Fig. 2). The water temperature in the experimental tank rose to 0°C on April 21 and above 1°C on May 14. The rate of loss of antifreeze from the plasma after winter is proportional to water temperature, with antifreeze being cleared from the system more rapidly at warmer temperatures (Fletcher et al. 1987). All the cod studied in this experiment were exposed to an identical water temperature profile. However, the cod from St. Lunaire lost their high levels of antifreeze rapidly sometime between April 3 and May 12 (at water temperatures of –0.5 and 0.8°C, respectively), while the cod from the three other sites showed little decline in antifreeze levels until after the May 12 sampling, at which point, a similar rate of decline was experienced by all four groups (Fig. 2).

Discussion

The results of this experiment confirm our hypothesis that antifreeze production capacity in Atlantic cod is related to overwintering environment. Perhaps our most significant finding was that cod from the most northerly location (St. Lunaire) were able to produce very high levels of antifreeze, sufficient to protect them from freezing under most conditions of ice and low temperature in the ocean. By midwinter, the St. Lunaire cod had about 50% greater freeze protection than juveniles from the three bays.

Our observations of antifreeze production in the four experimental groups of cod have allowed us to compare their physiological overwintering strategies and draw some conclusions as to the role played by AFGP in survival of juvenile cod in northerly environments.

The overall patterns of antifreeze production in terms of timing and AFGP levels (rising phase, mean maximum value attained, falling phase) were very similar in juveniles from Conception Bay, Trinity Bay, and Notre Dame Bay. No one group had experienced amplification of its antifreeze production capacity relative to the others, suggesting that these juveniles face similar overwintering conditions that demand about the same level of freeze resistance for survival. It also appears that antifreeze production capacity is stable over time, since the antifreeze levels reported for Conception Bay juveniles in this experiment are comparable with those found in juveniles in the same length range caught in Conception Bay during the summer of 1986, held at ambient temperatures over winter, and sampled in March 1987 (Kao and Fletcher 1988).

The Trinity Bay juveniles displayed less variation as a group and were more homogeneous in terms of maximum TH capacity than cod from the other three areas (Fig. 3). At maximum levels, the TH values found in Conception Bay, Notre Dame Bay, and St. Lunaire cod were spread out over a 0.7°C range, while the Trinity Bay juveniles' TH levels were all between 0.4 and 0.7°C (a range of 0.3°C). The same result was obtained when only fish from each group that fell within the exact length range of the Trinity Bay juveniles were compared (Fig. 4).

There is a considerable body of literature suggesting that, in addition to summer migrants from offshore, a discrete population of Atlantic cod lives year-round in Trinity Bay and that these resident cod spawn inshore in June–July (Goddard et al. 1994; Ruzzante et al. 1996; Smedbol and Wroblewski 1997). The juveniles collected from Trinity Bay may be the progeny of this resident spawning stock. Their greater homogeneity of maximum antifreeze production capacity could be related to their potentially discrete origin within Trinity Bay. The greater variation in maximum antifreeze production capacity observed in the juveniles collected from Conception Bay and Notre Dame Bay may be an indication of more diverse origins and, as such, may be more representative of the antifreeze production capacity of the majority of juveniles developing in coastal waters of northeast Newfoundland.

The rate of loss of antifreeze during the falling phase of the antifreeze production cycle was very similar in the Conception Bay, Trinity Bay, and Notre Dame Bay juveniles, with levels holding steady until May 12 and falling thereafter. However, the St. Lunaire cod lost about 26% of their total antifreeze activity between April 3 and May 12 (Fig. 2). Rate of postwinter antifreeze loss is temperature dependent in Atlantic cod (Fletcher et al. 1987). In this study, water temperatures rose above 0°C on April 21 and above 1°C on May 14. Between May 12 and June 28 (when water temperatures had reached 5.1°C), all groups lost antifreeze at the same rapid rate. It would appear that the pathways involved in clearing antifreeze from the plasma were able to function rapidly at temperatures below 1.0°C in the St. Lunaire juveniles, while in the other three groups, only temperatures >1.0°C resulted in antifreeze clearance. Such a downward shift in plasma antifreeze clearance temperatures could be a physiological adaptation to life at high latitudes. Historical (1962–1987) ice edge data show that the tip of the Great Northern Peninsula and coastal Labrador are generally icebound for about 1 month before and 1 month after other coastal areas of Newfoundland (Drinkwater et al. 1998). Such extended ice cover, reduced insolation, and delayed

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summer warming suggests that coastal waters from the tip of the Great Northern Peninsula northward could stay cold well into the summer although posing no danger of freezing to the juvenile cod living in these areas. Antifreeze proteins have been shown to significantly increase the viscosity of aqueous solutions and blood at physiological concentrations (Eto and Rubinsky 1993; B. Rubinsky and G.L. Fletcher, unpublished data). This study has shown that the winter antifreeze levels in the St. Lunaire juveniles are very high indeed. However, the persistence of high plasma concentrations of AFGPs during periods when there was no danger of freezing might be detrimental. Thus, for these fish, a physiological adaptation resulting in the clearance of antifreeze at lower temperatures could be highly advantageous, since it would result in a reduction of the work load on the heart associated with pumping viscous blood systemically, possibly coinciding with increased foraging activity in spring.

Our original hypothesis was that AFGP production capacity would be related to population origin coupled with the severity of environmental conditions experienced over the winter. Of the four groups examined, the St. Lunaire juveniles are likely to have been spawned farthest north, and their overwintering environments are likely to be the most severe, for the following reasons. The geographic spawning origin of St. Lunaire juveniles can be inferred from previous studies on distribution of spawning cod, egg and larval drift, and movements of cod juveniles. Offshore spawning of cod in southeastern Labrador has been reported to occur primarily at the outer edges of the continental shelf (Hutchings et al. 1993; deYoung and Davidson 1994). Hutchings et al. (1993) also reported evidence for inshore spawning at the tip of the Great Northern Peninsula in the location where the St. Lunaire juveniles were collected and in coastal areas further north along the Labrador coast (Black Bear Bay and St. Lewis Sound). There is general agreement that products of spawning off the coast of Labrador and northeast Newfoundland will either be retained in the area of spawning or be advected in a southerly direction by a component of the Labrador Current (Davidson and deYoung 1995). Anderson and Dalley (1997) suggested that pelagic juveniles found offshore will have originated primarily from spawning events upstream on the shelf, while pelagic juveniles found inshore will have originated primarily from inshore spawning (although some mixing may occur). Pepin and Helbig (1997) presented a range of possibilities including two-way transport of eggs and larvae between offshore (shelf) and coastal areas. Pepin and Helbig (1997) also suggested that Hamilton Bank and areas further north may represent important spawning grounds for eggs and larvae that transit through the coastal zone. From these reports, we conclude that eggs and larvae developing off the northeast coast of the Great Northern Peninsula could be the product of (*i*) a local spawning event, (*ii*) inshore spawning further up the Labrador coast, or (*iii*) an offshore spawning event further north, followed by transport of eggs and larvae into the coastal region.

However, the cod used in this experiment were probably 2–3 years old and thus capable of significant movement. Studies of movement patterns of juvenile cod during their first years of life suggest that there is considerable variation in the geographic range covered and the degree of fidelity to nursery grounds (Clark and Green 1990). Using ultrasonic telemetry to track 3-year-old cod in Conception Bay from June to December, Clark and Green (1990) found that while there were diel and seasonal variations in activity patterns and range of travel, the distances covered were relatively short, and these juvenile cod did not migrate away from their home range within Conception Bay. Juveniles do not appear to join the main migrating schools of cod in the northeast Newfoundland and Labrador region until they approach maturity (40+ cm in length) (Rose 1993). Since Bogstad et al. (1994) reported that 40 cm is the approximate cutoff point for predation of adult cod on juveniles, it appears that this is the safe size at which juveniles can join the migrating adults without risk of being eaten and reap the benefits of the migratory shoaling way of life. Thus, while age-3 juveniles may be capable of considerable dispersal from their nursery sites, it is also quite possible that they will remain in loose association with their nursery grounds until nearing sexual maturity, at which point, they embark on the more extensive migrations carried out by the adults (Rose 1993). Thus, we have assumed that the juveniles collected from St. Lunaire were representative of northerly (high-latitude) spawning, probably inshore, but possibly further afield.

The four sites from which cod were collected are all at the northern end of the distributional range for Atlantic cod (Leim and Scott 1966), but the St. Lunaire site appears to present the greatest and most temporally extended danger in terms of potential for ice contact at temperatures below the plasma freezing point. Each winter, the area is ice-bound for considerably longer than the other three sites, up to 2 months longer in some years (Symonds 1986; Drinkwater et al. 1998). Ice cover affects the underlying environment in several ways, all of which appear to be detrimental to the survival of teleost fish, since they increase the chances of exposure to the lethal combination of ice and temperatures below the plasma freezing point. For example, the proportion of incident solar radiation capable of heating up the surface waters is substantially reduced by ice reflection when the sea is ice-covered and by ice melt as the ice retreats; during surface ice growth, the exclusion of salts from the forming ice can produce density gradients that result in vertical mixing of the freezing surface waters down to considerable depths (Narayanan et al. 1995). The AFGPs are functional compounds offering protection against freezing and cold damage, and the degree of freeze protection that they confer is directly proportional to their concentration in the body. Thus, while harsh environmental conditions at all sites have resulted in selection for cod populations capable of producing high winter levels of antifreeze, the greatest AFGP production capacity is found in the population inhabiting the most extreme winter environment.

Two hypotheses might be invoked to account for the greater antifreeze production capacity of the St. Lunaire population. It could be the result of short-term selection for the most cold-resistant individuals from a whole suite of cold tolerance levels in a panmictic population. It could be indicative of a population that has evolved over time, in response to environmental pressure, to have greater antifreeze producing characteristics than cod occupying somewhat less extreme environments.

The first option, that the observed population differences are the result of differential survival, with only the more

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cold-resistant individuals surviving to age 3 at the most northerly site, appears unlikely for the following reason. By early April, 11 of the 32 fish from St. Lunaire had attained TH levels ≥1.0°C and plasma freezing points comparable with that of the ocean (≤–1.8°C). None of the juveniles from the other groups (N = 107 animals) developed this level of freeze resistance. Thus, an explanation other than selective mortality seems more appropriate.

Population differences in antifreeze production capacity have been found in other northern teleost species that have a wide geographic distribution and a greater need for cold protection at high latitudes. Both winter flounder (*Pleuronectes americanus*) and ocean pout (*Macrozoarces americanus*) from the Newfoundland region are capable, under the same environmental conditions, of producing significantly higher levels of antifreeze over a winter cycle than their counterparts from more southerly areas of the distributional range (Fletcher et al. 1985*a*, 1985*b*). For both these species, the molecular tools necessary for investigating differences in antifreeze gene dosage have been developed, with the following results. Genomic Southern blotting has shown that within the Newfoundland ocean pout genome, the number of antifreeze protein genes is about four to five times greater than the antifreeze gene dosage in the New Brunswick ocean pout (Hew et al. 1988). Investigations into the genomic organization of liver-type antifreeze genes in the winter flounder have revealed an antifreeze multigene family of about 40 members, about two thirds of which are arranged as direct tandem repeats, the result of amplification of the antifreeze gene sequence (Davies et al. 1984; Scott et al. 1985, 1986). It has been shown that the degree of amplification of the antifreeze protein genes (i.e., the gene dosage) can be correlated with the level of antifreeze produced and with the environmental freezing conditions to which the fish are exposed (Fletcher et al. 1998). The winter flounder, with its tandemly amplified antifreeze genes, is able to produce at least two times more antifreeze protein than the closely related yellowtail flounder (*Pleuronectes ferrugineus*) over a winter season. While both species possess the basic complement of about 10 linked but irregularly spaced antifreeze protein genes, only the winter flounder has the tandemly linked antifreeze protein gene grouping (Scott et al. 1988; Fletcher et al. 1998).

Amplification of AFGP gene dosage would be an advantage when colonizing the more extreme northerly environments. Could this be the underlying explanation for the greater freeze resistance of the cod population from the Great Northern Peninsula?

Studies with *Notothenia coriiceps neglecta* (an Antarctic teleost that also produces AFGP) have revealed the presence of an AFGP polyprotein gene encoding 44 copies of AFGP 8 and two of AFGP 7, which are produced together as a high copy number polyprotein and processed posttranslationally to produce active AFGP (Hsiao et al. 1990). Only AFGP 7 and AFGP 8 are represented in this gene, and thus, other genes must be present in the fish genome to encode the remaining relative molecular mass forms, suggesting that the AFGPs are encoded by a multigene family (Hsiao et al. 1990). Activity studies on AFGP forms 1–8 reveal that the larger glycoprotein molecules (1–5) are more effective at depressing the freezing point than the small ones (7 and 8) (DeVries and Lin 1977). These facts suggest two mechanisms whereby the freeze-depressing capacity within an individual or population could be increased: overall gene amplification resulting in higher plasma AFGP levels or selective amplification of the genes coding for the AFGP components with highest activity. Although hypotheses can be advanced to account for the greater capacity for cold resistance in the juveniles collected from the most northerly site, these questions cannot at present be resolved due to the lack of appropriate molecular tools. However, the possible genetic basis for population differences in antifreeze production in Atlantic cod would prove an interesting topic for future study.

In conclusion, we have shown that Atlantic cod living off the Newfoundland and Labrador coast are physiologically adapted to tolerate environmental conditions of low temperatures and ice and that those cod inhabiting the more demanding overwintering environments at the more northerly end of the historical range have developed patterns of antifreeze production that match that demand. It seems likely that such physiological adaptations to extreme environments will play a role in the development of stock structure in species such as the Atlantic cod that occupy a very wide distributional range. In recent years, the northern cod stock has become severely depleted throughout its range, but particularly in the north. It is possible that discrete populations that have evolved over time to tolerate the more extreme conditions experienced at high latitudes have been removed from the system or reduced to very low numbers. This may have an impact on the rate at which the northerly reaches of the cod's historical distributional range can be recolonized.

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Sonar estimates of daytime activity levels of *Euphausia pacifica* in Saanich Inlet

Jules S. Jaffe, Mark D. Ohman, and Alex De Robertis

Abstract: A three-dimensional multibeam tracking sonar system (FishTV) was deployed in the stratified waters of Saanich Inlet in July–August of 1996 and 1997 to assess the swimming behavior of euphausiids in situ. Here, a new algorithm is used to estimate swimming velocities of animals from the uncorrelated displacements of acoustic targets in pairs of sonar frames with increasing time delays between frames. Assuming isotropic motions of euphausiids, the superior spatial resolution of the sonar in one dimension (range) is used to infer the three-dimensional motions. We applied the algorithm to sonar tracks of groups of 10 000 – 25 000 euphausiids recorded in the daytime at depths just above the oxycline on six different occasions. The animal movements were described with a probability density function for velocity which suggests that about 60–70% of the animals were moving less than or equal to 0.3–1.2 cm·s^{-1}. These distributions imply remarkably low swimming speeds and quiescent behavior. Limited motility in the daytime would confer two major advantages on the euphausiids: reduction of encounter rates with ambush predators and decreased metabolic costs.

Résumé : Nous avons déployé un système de pistage tridimensionnel par sonar multifaisceaux (FishTV) dans les eaux stratifiées du bras Saanich en juillet-août 1996 et 1997 pour examiner in situ le comportement de nage des euphausiacés. Nous utilisons ici un nouvel algorithme pour estimer les vitesses de nage des animaux à partir des déplacements non corrélés de cibles acoustiques dans des paires de clichés sonar en faisant croître l'intervalle entre les clichés. En supposant que les déplacements des euphausiacés sont isotropes, nous nous sommes servis de la résolution spatiale supérieure du sonar dans une seule dimension (la portée) pour inférer les déplacements dans les trois dimensions. Nous avons appliqué l'algorithme aux tracés sonar de groupes de 10 000 à 25 000 euphausiacés enregistrés à six occasions différentes, de jour, à des profondeurs juste supérieures à l'oxycline. Les déplacements des animaux ont été décrits avec une fonction de densité pour la vitesse qui permet de penser que 60 à 70% des animaux se déplaçaient à une vitesse inférieure ou égale à 0,3–1,2 cm·s^{-1}. Ces distributions correspondent à des vitesses remarquablement basses et à un comportement passif. Leur motilité réduite pendant le jour peut conférer deux grands avantages aux euphausiacés : réduction des taux de rencontre avec des prédateurs à l'affût, et baisse des dépenses métaboliques.

[Traduit par la Rédaction]

Introduction

Euphausiid crustaceans are distributed throughout the World Ocean (Brinton et al. 1999) and in many localities are dominant components of marine food webs. Owing to their relatively large body size, they are disproportionately significant as prey items for a variety of marine planktivores. Quantitative models of euphausiid bioenergetics, encounter rates with prey and predators, bioenergetics, and diel vertical migrations are dependent upon knowledge of a variety of behavioral characteristics, most of which remain poorly known. None have been determined for individual euphausiids in their natural habitat. In this article, we report measurement of the daytime activity level of *Euphausia pacifica* at depth in Saanich Inlet, British Columbia. To our knowledge, this study is the first quantitative assessment of the swimming speeds of individual euphausiids in situ.

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The ability to formulate sensible models for prey–predator encounter, individual somatic growth, and growth of populations rests upon knowledge of natural behavior. For example, among the more important parameters for encounter models are prey and predator swimming velocities and local prey densities (e.g., Gerritsen and Strickler 1977; Buskey et al. 1993). Therefore, diel variations in swimming velocity and in formation and dispersion of local prey patches can markedly affect computations of encounter rates. Similarly, bioenergetic growth models require accurate knowledge of metabolic losses. Since the metabolic rate of euphausiids is known to depend steeply upon the swimming speed of the animals (Torres and Childress 1983), it is essential to understand natural swimming speeds and activity levels of euphausiids. Some models assume negligible swimming speeds of euphausiids when they are at daytime depths (Andersen and Nival 1991), but this has never been measured and remains an important unknown quantity.

With the recent development of FishTV (Jaffe et al. 1995) and the combined OASIS (optical–acoustic submersible imaging system) system (Jaffe et al. 1998), we now have the capability for both tracking and identifying individual zooplankton in situ. The system can be used to measure the abundance of zooplankton by counting the number of reflec-

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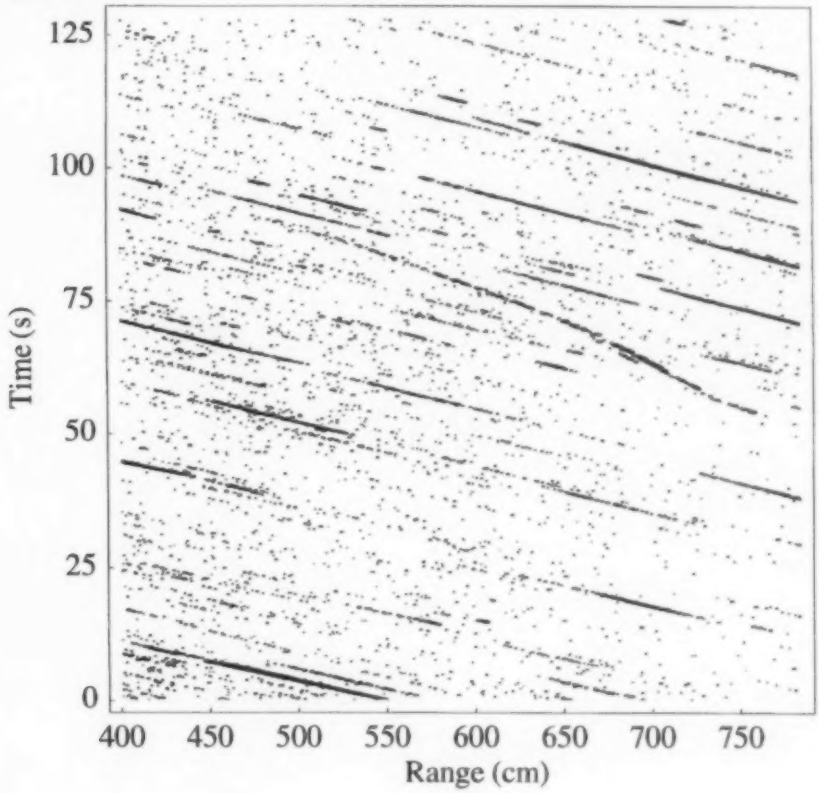
Fig. 1. Image created by averaging the acoustic returns from the inner four beams of the sonar system as a function of range and time. The data were contoured as a fraction of the maximum target strength (about –67 dB) with the black values corresponding to targets that were within 5% of this value.



tions in the insonified volume from individual animals. The intensity of the reflections can then be used to obtain the animals' target strength. Three-dimensional locations of animals are determined that can then be linked together in successive frames to form three-dimensional trajectories (McGehee and Jaffe 1996). Briefly, the OASIS system consists of a sensitive CCD camera, a set of current meters, and the FishTV system, mounted on a large current vane that is oriented so that in even moderate current (1–5 $cm \cdot s^{-1}$), the sonar is pointing into the current. Observations of animal trajectories indicated that there was no detectable behavioral response to the presence of OASIS (Jaffe et al. 1998), as the device was downstream from the animals and data collection started at a range of 2 meters from the transducers.

As a study site for our investigations, we chose Saanich Inlet in British Columbia. Saanich Inlet is a fjord with an oxygenated surface layer and seasonally anoxic bottom waters, with a large resident population of *E. pacifica* (Boden and Kampa 1965). In midsummer, horizontal advection and vertical shear are relatively modest, apart from episodes of strong winds and tidal mixing associated with spring tidal cycles. Visual observations (Mackie and Mills 1983) from the *Pisces IV* submersible in Saanich Inlet indicated that *E. pacifica* aggregates immediately above the oxycline.

Methods and materials

Experimental considerations

These studies were conducted in two consecutive summers (1996 and 1997) during 2 weeks in late July and early August. An initial CTD-O_2 survey along the central axis of the fjord was used to measure the depth of the anoxic layer. Based on these measurements, a site was chosen (48°34.4′N, 123°30.4′W) where euphausiids aggregated at a depth of about 70–90 m during the daytime. This depth was chosen to be slightly shallower than the present operational depth capability of the OASIS system. The OASIS system, which was deployed in both a vertical profiling mode and at several fixed depths, provided vertical distribution patterns of euphausiids that were consistent with those derived from MOCNESS tows (A. De Robertis, unpublished data).

Briefly, the FishTV system is a multibeam sonar system that operates at a frequency of 445 kHz and at frame rates of up to 4 Hz. The system provides acoustic backscatter from a set of 64 beams whose beam widths are 2 × 2°. The system resolves 512 range bins at a range increment of 0.75 cm, which yields a three-dimensional data set of dimensions 16° × 16° × 3.8 m. Extensive target strength determinations done recently with live euphausiids (A. De Robertis, unpublished data) indicate that, on average, the system can resolve animals down to a body length of 7–10 mm under the system parameters used for this set of observations.

The present study is based on six extended deployments at a

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Fig. 2. Simulation of the trajectories of 100 targets as a function of time. The targets were assigned uniformly distributed starting positions initially in a 10 × 10 unit area and then translated by an amount that was the superposition of a uniform current to the right and a normally distributed velocity distribution whose standard deviation was 0.25 distance unit per time interval. Shown are the positions of the targets at delays of (*a*) one, (*b*) two, (*c*) three, and (*d*) four time intervals (ΔT) and (*e*) the underlying probability density functions for each set of horizontal movements.

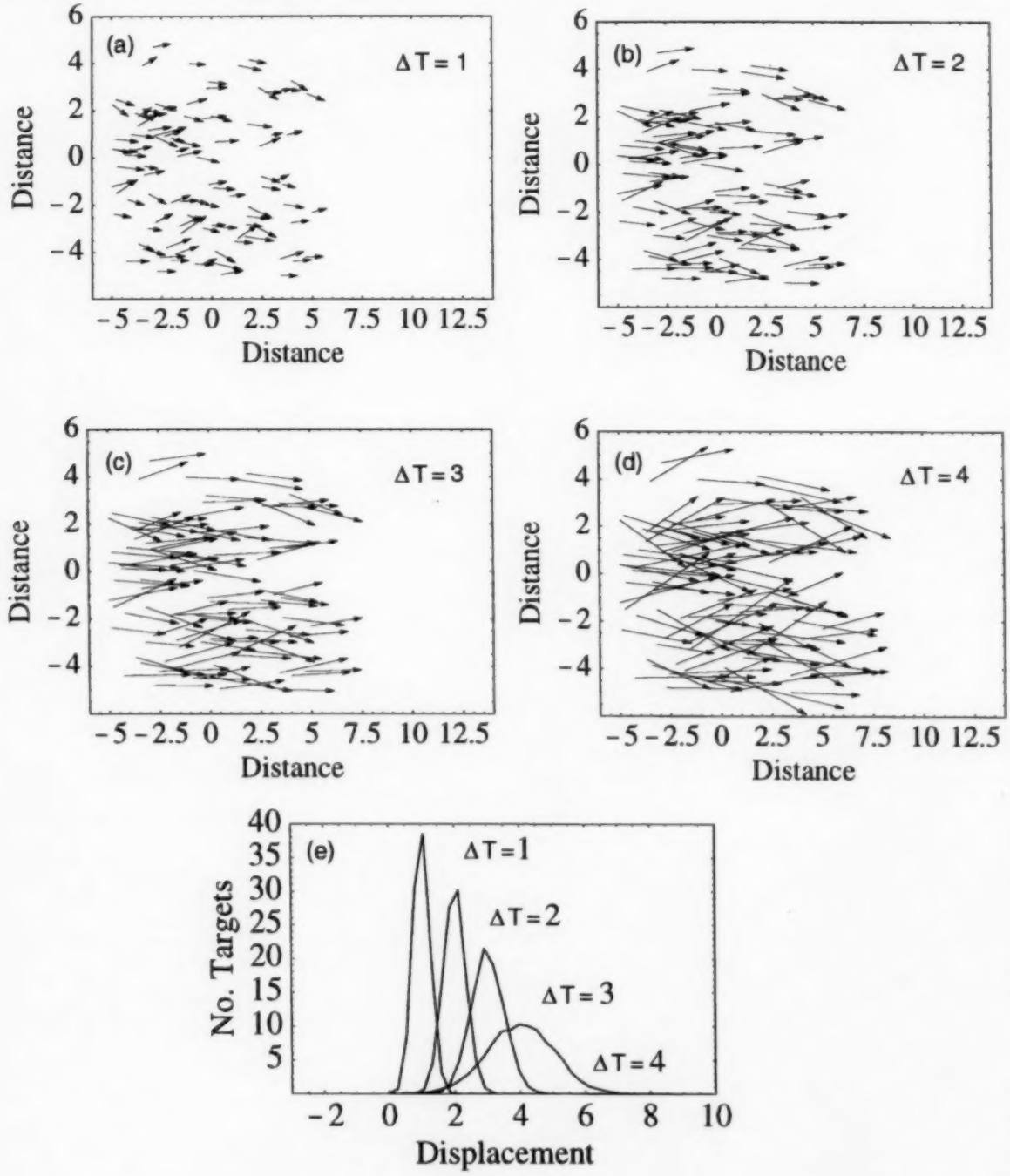

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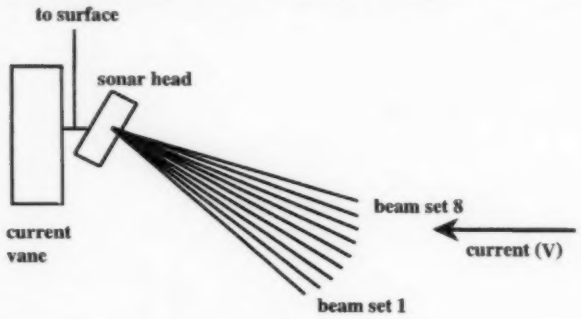
Fig. 3. Schematic illustration of the geometric arrangement of the FishTV transducers and current vane during deployment. Each projected beam is received by eight transducers.



fixed depth in the daytime aggregation layer, four in 1996 and two in 1997 (see Table 1). Several thousand frames of animal sound reflection were collected in each session with up to hundreds of animals in each. The OASIS system was deployed at a stationary depth of 70–90 m for intervals of 5–30 min during the daytime. For this study, a set of six runs were used, four from the summer of 1996 and two from the summer of 1997. During 1996 the sonar was operated at a rate of 2 Hz and in 1997 at 4 Hz. During 1996 the sonar acquired data starting at a range of 1.9 m and ending at a range of 5.7 m. In 1997, the sonar acquired data starting at a range of 4 m and ending at a range of 7.8 m. We chose to use only a subset of these animal trajectories, the strongest 20 acoustic targets in each frame, to insure that the same individuals were detected in successive frames. The number of animals that were tracked was a decreasing function of the length of observation time, with about 25 000 animals tracked for the shortest time intervals (0.25–0.5 s) to about 10 000 animals tracked for the longest intervals (2–4 s).

Mathematical considerations

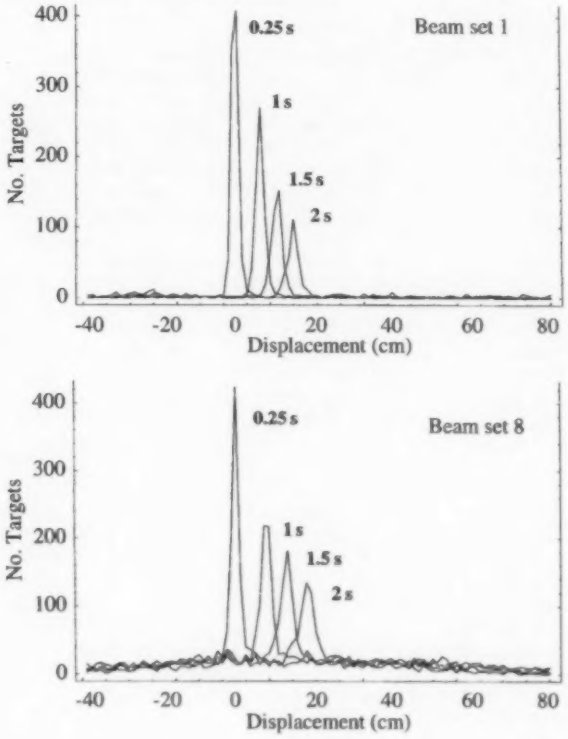
Consideration of the sonar geometry and its anisotropic resolution in three dimensions motivated the development of a special algorithm to detect small motions. Both the motivation for this algorithm and the theoretical basis for its performance are considered in this section.

As an example of the problem encountered, consider that the azimuthal resolution of the system is 2 × 2°. At a range of 3 m, this results in a resolution of 10.5 × 10.5 cm across the beam. In contrast, the range resolution of the system, which is dictated by the bandwidth of the signal, is about 1 cm. Since the animal motions were suspected to be small (see below), an algorithm was developed that makes use of this much greater range resolution.

An image containing the tracks of many animals is illustrated in Fig. 1. It was derived by taking the range-varying intensity of the average backscatter of the inner four acoustic beams and displaying it as a function of time. The figure shows many dark diagonal lines of high acoustic reflectivity that can be interpreted as animals that are drifting towards the device. Since the sonar is pointed into the current, the drift is at a constant rate. The parallel lines in Fig. 1 suggest that the observed motions are primarily due to drift and not due to animal swimming. On the other hand, several trajectories show animal motions that are not "going with the flow" and can be interpreted as swimming. Animal trajectories also seem to appear and disappear. Whether these starts and ends are due to the animals going outside the inner four beams or perhaps the animals changing their orientation and thus decreasing their acoustic reflectivity cannot be distinguished from this analysis.

It therefore seemed reasonable to assume that the animals' motion relative to the sonar system could be regarded as a mean displacement, due to the mean current, $<\vec{v}>$, plus some small

Fig. 4. Set of displacement distribution functions (the number of targets that underwent translation Δx in time interval ΔT as a function of time delay and beam set for the data collected on 31 July 1996. (*a*) Beam set 1 is the set of eight beams that are pointing most downward and (*b*) beam set 8 is the set of beams that are oriented the most horizontally.



changes, $\Delta\vec{v}$, due to a combination of the animals' movements and turbulent motions so that the total movement is $<\vec{v}>+\Delta\vec{v}$. Figure 2 shows the results from a simulation designed to illustrate the basic concept behind this method. The figure depicts the trajectories of 100 particles whose velocities are the sum of a uniform component and a random component. The starting position of each particle was chosen so that the particles were uniformly distributed in a 10 × 10 unit area centered on the origin. Next, each particle was displaced by an amount that was the superposition of a uniform flow to the right and a random component. The uniform flow was 1 unit per time interval and the random component was normally and isotropically distributed in the plane with a standard deviation of 0.25 unit per time interval. A single random component displacement was chosen for each particle, evaluated at $\Delta T = 1$. This same random displacement was then applied four times to each particle (once for each time delay) and added to the uniform flow. The four vector figures (Figs. 2*a*–2*d*) show the displacements of the particles (from $T = 0$) at different time intervals. Figure 2*e* depicts a historgram of the total particle displacement (relative to $T = 0$) as a function of delay time for the set of 100 particles. The translation of the peak to the right is due to the mean current, while the spreading of the peaks with increasing time delay is due to the uncorrelated motions of the targets and thus estimates the animals' true swimming velocities.

Because movement of the animals can be most accurately measured in the range direction, a technique was developed to obtain a statistical characterization of the animals' three-dimensional mo-

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tions from these range fluctuations after subtracting out the mean flow $< \bar{v} >$. Under the assumption that the animals' movements are isotropic, changes in range can be regarded as one component of a three-dimensional distribution function. The remainder of this section describes an algorithm that yields an estimate of this distribution function from the range data.

Mathematical algorithm

The algorithm described in this section was designed to yield an estimate for the probability density function for animal velocities, pdf(v). This function describes the animal population with respect to the motion of the animals under the assumptions of temporal and spatial stationarity over the observation time. The procedure is simplified by assuming that the function does not change as a function of orientation, i.e., it is isotropic. Therefore, every one of the individual sonar beams should be measuring approximately the same displacements. By tracking animal displacements in the same beam for different time intervals, a displacement distribution function is obtained, $\mathrm{ddf}_1(\Delta x,\Delta T)$, which is the number of animals that underwent displacement Δx for a fixed delay time ΔT. We use the notation "ddf" to denote that this function is not strictly a probability density function. Under the assumption of isotropy, there is a straightforward relationship between this function and the true three-dimensional displacement distribution function $\mathrm{ddf}_3(\Delta x,\Delta T)$, which can be used to estimate this true distribution function from the measured data for each time delay. Although this function could then be used to directly estimate the pdf(v), a slightly different procedure is used here to obtain this function. Instead, the rate of spreading of this displacement function yields a more accurate estimate for the animal movements than the observation of the individual displacement distribution functions because it takes into consideration the accuracy of the system in localizing animals. Using the set of time-delayed displacement functions, the standard deviation of each one is computed as an estimation of the width of the peak, and then, a straight line is fitted to the set of standard deviations as a function of time delay. The slope of this line, equal to the rate of the spreading of the standard deviation, provides a measure of the function width for pdf(v).

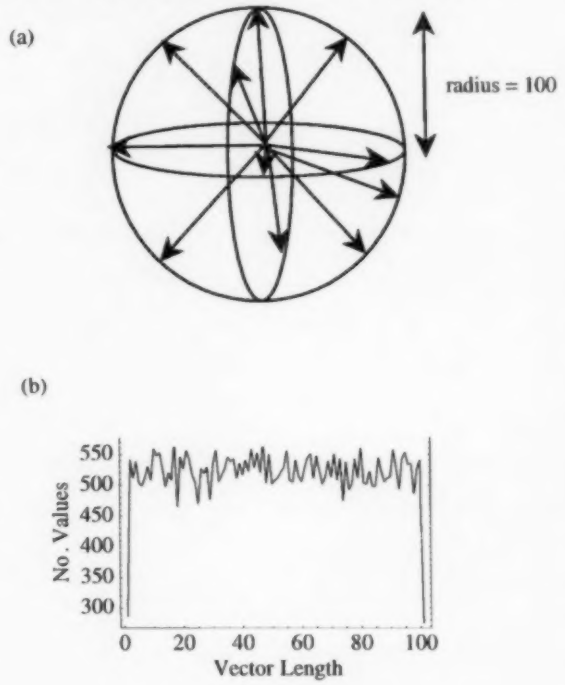
The starting data for the estimation procedure is a set of matrices of sonar backscatter intensity from a single deployment. We use a procedure called "trimming" to identify regions of local maximum backscatter and then trim out the surrounding area, which can have large values of backscatter due to the side lobes of the reflected sound. The procedure is as follows: first, the backscatter matrix is inspected to find its maximum value. Next, after applying a correction for range spreading, attenuation, and calibration of the system, the target strength of this maximum value and its location in three dimensions are computed. Since the beam patterns from our system result in some spreading of the sound into the adjacent volume (mostly in the azimuth), the volume immediately surrounding the identified target is excluded from consideration in finding more targets. The subsequent matrix is inspected for the next highest value and this procedure is repeated until a user-specified target strength (lower bound) is reached. At the end of this procedure, a list of target positions and target strengths is available as a function of frame number for a single set of acoustic reflections.

The list of target strengths is then truncated to the 20 strongest targets per frame that are compatible with measured target strengths of adult euphausiids from the OASIS system (Jaffe et al. 1998). For the most part, these target strengths are associated with adult animals of 15–20 mm total length (–78 dB < TS < –71 dB).[2] Using only the largest animals increased the chances of detection from frame to frame while minimizing the chances of mixing different animals in consecutive positions in sequential frames. These truncated target lists were then processed to obtain an estimate for a one-dimensional probability distribution function of displacements as a function of delay time, $\mathrm{ddf}_1(\Delta x,\Delta T)$, by computing the changes in range between all of the sets of 20 targets between frames for a given time delay ΔT.

To increase the probability that the same animal is being considered, the displacement values were used only if the target stayed in the same beam for the pair of frames that were under consideration. These difference matrices were integrated over all of the frames for a single set of data and then binned to provide the number of targets that underwent displacement Δx for time interval ΔT. At the end of this stage of processing, a set of one-dimensional displacement distribution functions for displacement as a function of delay time were obtained.

The geometric arrangement for the deployment, showing the grouping of the 64 beams into eight sets of eight beams, is illustrated in Fig. 3. Beam set 8 was oriented the most horizontally (declined at 19° from horizontal) and beam set 1 was oriented the most vertically (declined at 33°). The density displacement functions $\mathrm{ddf}_1(\Delta x,\Delta T)$ for beam set 1 and beam set 8 for one of the runs are shown in Fig. 4. The figure illustrates several features of the data sets as follows. (*i*) The mean displacement of the peak increases with time delay between frames due to the mean current. The slope of the relationship between mean displacement and time

Fig. 5. Simulation illustrating the relationship between a set of 50 000 three-dimensional translations and the actual one-dimensional projections that are measured by the system. (*a*) The three-dimensional translations are all of the same magnitude, x', however isotropically oriented. (*b*) Values that would be measured by projecting this set of three-dimensional vectors onto a single one-dimensional vector, corresponding to the actual range measurements that the sonar would be making.



[2] Our previous paper, which documented the first use of the OASIS system (Jaffe et al. 1998), contains a calibration error: all target strengths reported in that paper were overestimated by 6 dB.

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Table 1. Estimated swimming speeds of *E. pacifica* from six deployments in Saanich Inlet, B.C.

Date	Time	Depth (m)	Estimated swimming speed ($cm \cdot s^{-1}$)	SD ($cm \cdot s^{-1}$)
29 July 1997	18:10–18:13	80	0.3	0.23
31 July 1997	16:05–16:09	80	0.9	0.40
8 August 1996	10:11–10:17	80	1.0	0.21
12 August 1996	10:49–10:57	85	1.2	0.43
13 August 1996	11:07–11:15	90	1.1	0.28

Note: Swimming speeds are estimated from the rate of spreading of animal displacement distributions. Standard deviations of swimming speeds are estimated from numerical simulations that computed the resultant standard deviations in the estimated swimming speeds by performing the linear inversion process 10 000 times using the observed standard deviation among the beam sets as an estimate for the true standard deviation of the data.

delay approximates the mean current speed. (*ii*) The width of the peak increases as a function of delay time, due to the dispersion in the measured positions of the animals. This spread in the peak width approximates the swimming speed of the animals. (*iii*) The beam set pointing most horizontally, beam set 8, undergoes a larger mean displacement than beam set 1 because the horizontal projection of the mean current vector was slightly larger than for beam set 1. (*iv*) The widths of the distribution functions for the more horizontally oriented beams were slightly wider than for the more vertically oriented ones (this point will be discussed in more detail below).

The next step was to compute an estimate of the three-dimensional displacement distribution functions, $ddf_3(\Delta x,\Delta T)$, from the one-dimensional displacement distribution function, $ddf_1(\Delta x, \Delta T)$, by applying a linear transformation. Although the details of the transformation are provided in the Appendix, the motivation for its use is described here. As an example of one particularly peculiar case, consider a center point of location {0,0,0} as shown in Fig. 5 and the simulated set of 50 000 isotropic displacements of fixed distances $\Delta x'$. Because the methodology here makes use of only the range dimension, the relationship of the range data to the true three-dimensional data needs to be considered. The set of three-dimensional locations $\{x_i,y_i,z_i\}$ are "projected" onto the axis of the sonar beam, yielding a set of displacement distances (Fig. 5*b*). The figure illustrates the somewhat curious result that the projection of the set of three-dimensional displacements of an isotropic and single-valued displacement onto one axis results in a constant function. Theoretical analysis of the problem (not presented here) validated this numerical result. Evidently, the result of projecting this three-dimensional distribution onto a one-dimensional beam direction has resulted in a distortion of the probability density function. Since this projection operator is essentially an inner product between the displacement vectors and the beam direction, it is a linear transformation. Moreover, since any one-dimensional observed distribution is a sum of a set of these simple displacement distributions, a linear transformation can be formulated that describes the forward problem. In principle, inversion for the true three-dimensional distribution function from these one-dimensional observations can then be performed. The Appendix contains a proof that the animal displacements are approximately a projection of the displacements, as well as an example of the projection of a normal distribution and an explanation of the inversion technique that was used to obtain an estimate of the three-dimensional displacement density functions from the one-dimensional observations. Four examples of the transformation from $ddf_1(\Delta x,\Delta T)$ to $ddf_3(\Delta x,\Delta T)$ are displayed in Fig A2 in the Appendix.

Although the resultant three-dimensional displacement probability density functions could simply be averaged together to obtain an estimate for the velocity distribution (after dividing through by time), an additional step was added to provide a more accurate estimate of the animal motion. The motivation for its implementation comes from the following reasoning: even though the system can measure range accurately, the measurement of displacement is still subject to a myriad of environmental effects. Fluctuations in animal orientation for animals that subtend more than one range bin, rotation of the sonar system about the suspension wire, and other small instrumental motions are all examples of factors that can degrade the range displacement resolution of the system. Any spreading of the displacement distribution as a function of time would then be superimposed on these more systematic changes. For this reason, the approach taken here attempted to estimate and subtract these motions.

To obtain an estimate for the displacement resolution of the system, the peak widths of the set of three-dimensional displacement distribution functions were computed. Next, a linear fit of the peak width as a function of time delay was obtained. Both the slope and the intercept were computed. The slope of this curve, equal to the value at a 1-s delay with the systematic delay subtracted off, was then used to estimate the true peak width of the animal probability density function for velocity. So, for example, if the measured peak width is pw_{meas} and the true peak width is pw_{true}, then the effect of animal motion leads to a linear spreading of this distribution so that

$$pw_{meas} = pw_{spread\ rate} \times time + sd_{offset}$$

where sd_{offset} is the intercept of the linear function at time delay zero. The slope at 1-s delay is thus equal to $pw_{meas} - sd_{offset}$, which is assumed to approximate the true animal distribution (pw_{true}). So, for example, if the animals were not moving but the instruments were still subject to the environmental effects, a set of identical displacement probability density functions of some finite width would be observed. In this case, since the standard deviations of the set of displacement probability density functions would not change, the slope of a plot of standard deviation versus delay time would be zero, leading to the correct inference of no animal movement. This procedure assumes that the broadening of the animal displacement function can be viewed as a linear process and that it makes sense to simply subtract out the extrapolated zero time delay value. A more complete treatment would likely involve more sophisticated linear inverse theory; however, the essential results of this analysis would probably not change.

Results

The acoustic returns from six sessions where the sonar was deployed at a fixed depth in the daytime were processed by the algorithms described above. These resulted in the estimation of animal positions in three dimensions, the computation of the one-dimensional displacement density functions, the inversion for the three-dimensional distribution functions, and an estimation of the width of those distributions (the latter approximated by their standard deviation). To address variability of estimated swimming speeds, the individual beam sets were not combined until the last stage of the data pro-

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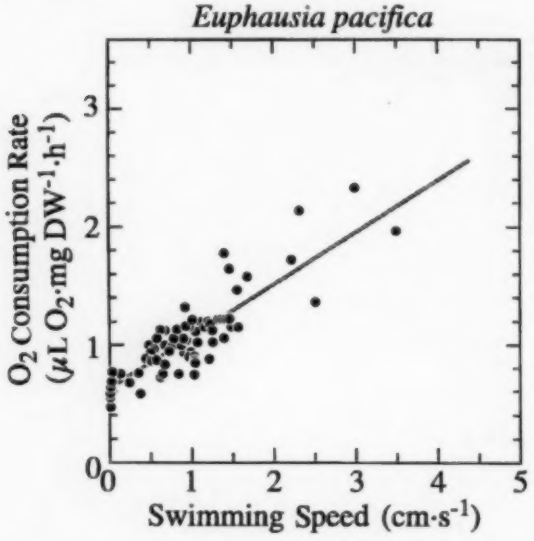
Fig. 6. Relationship between oxygen consumption rate and swimming speed for *E. pacifica* (redrawn from Torres and Childress 1983). Measurements were made at 8°C and 1 atm pressure (101.325 kPa) in the daytime, but animals were cycled to 12°C at night while held in the laboratory prior to experiments.



cessing. In this way, an estimation of beam-to-beam consistency of the spread in peak widths for a single run was possible.

The results from analysis of the six data sets are listed in Table 1. In all cases the average speed of the animals, as estimated by the standard deviation, was quite low. It ranged from 0.3 to 1.2 cm·s^{-1}. In order to estimate the variability associated with these mean speeds, a Monte Carlo approach was employed. First, the standard deviations for the set of peak widths were computed for a given delay time and deployment by comparing the peak widths from the eight beam sets. Next, random numbers were drawn from a normal distribution which used the observed mean peak width as an estimate of the true mean peak width and the observed standard deviations among the beam sets as an estimate of the true standard deviation of the estimated swimming speed estimates. Then, the slope of the line of peak width versus delay time was computed. This linear inversion was then repeated 10 000 times for each realization of the four peak widths as a function of delay time. These values are all less than the mean values, indicating relatively little variability among the beam sets in estimating swimming speeds. In addition, the intercept of the straight line fitted to peak width as a function of time delay estimated the systematic delay or the ability of the system to resolve animals positions. These values ranged from 0.02 to 1.2 cm.

Cumulative distribution functions were calculated for a few of the data sets. The purpose of this analysis was to see what fraction of the animals were moving at speeds within one standard deviation from the mean total displacement. Consistent with the distributions being approximately Gaussian, between 60 and 70% of the animals were moving less than or equal to this value.

Discussion

The most striking feature of the estimated swimming speeds of euphausiids reported here is their low values compared with previously reported values. Sustained pleopod swimming speeds of euphausiids from measurements made in aquaria (Hanmer 1984; Price 1989; Land 1992) and in situ estimates (Hanamura et al. 1984; M.D. Ohman, unpublished data) when scaled to body length range from 0.7 to 5 body lengths·s^{-1}. Because the swimming speeds observed in this study (0.5–1 body lengths·s^{-1}) are at the lower end of this range and are low in absolute units (0.3–1.2 cm·s^{-1}), we infer that *E. pacifica* exhibits quiescent behavior with limited motion in subsurface waters during the daytime.

While not surprising, these results provide the first direct evidence for markedly reduced swimming activity of diel migrant euphausiids at their daytime depths. This behavior has at least two adaptive advantages over the alternative of sustaining swimming activity in deeper strata. First, the reduction of swimming velocity will reduce encounter rates with most types of predators, especially ambush predators (e.g., Gerritsen and Strickler 1977). Second, reduced motility is an energetically efficient behavior that decreases metabolic costs.

This reduction in metabolic cost as a consequence of reduced swimming activity at depth should not be confused with once-hypothesized metabolic advantages of diel vertical migration behavior, a long-abandoned (McLaren 1974) argument for zooplankton diel vertical migration. Torres and Childress (1983), in the best experiments to date addressing the metabolic costs of swimming in euphausiids, discovered that there is more than a threefold increase in metabolic rate as *E. pacifica* increases its rate of swimming (see Fig. 6, redrawn from their study). Work on zooplankton metabolism prior to these authors often arrived at different conclusions because of the authors' failure to distinguish between basal metabolism (animals at rest) and routine metabolism (animals spontaneously active). When this distinction is made properly and resolved experimentally, the rate of oxygen consumption varies steeply with the activity level of the animals (Fig. 6), conferring considerable energetic benefit on quiescent behavior. Conversely, as Torres and Childress (1983) pointed out, the increase in swimming speed when animals migrate vertically results in substantial metabolic costs that nullify any hypothetical metabolic savings of diel vertical migration for euphausiids. The adaptive value of diel vertical migration for euphausiids, as for other zooplankton (Ohman 1990; Bollens et al. 1994), generally concerns the minimization of predation risk. Substantial energetic costs associated with locomotion have been demonstrated in planktonic mysids (Buskey 1998*a*), lophogastrids (Cowles and Childress 1988), and planktonic copepods (Buskey 1998*b*).

The velocities that we have measured from the rate of spreading of animal displacements as a function of time delay are the uncorrelated velocities between pairs of acoustic reflectors. These velocities actually include two sources of motion: the swimming motions of the animals and an unknown contribution of turbulent fluid motions. We are not able to resolve the relative contributions of these two sources. However, our result is not affected by this ambiguity. In the extreme case of no turbulent motions, all of the observed displacement would be caused by swimming,

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which leaves the motion remarkably small. In the other extreme, if all the motion were due to turbulent velocity fluctuations in the fluid, the animals would be absolutely stationary in the daytime, further reinforcing our result.

We tested our assumption of isotropic swimming motions by analyzing three-dimensional swimming vectors of euphausiids using a target-tracking algorithm. These results showed that the euphausiids in this study site exhibit nearly isotropic swimming behavior at most times (A. De Robertis, unpublished observations). An exception occurs at the times of peak vertical flux during diel vertical migrations, when animal displacements are highly directional, but such intervals are not relevant here. Furthermore, any small departure from isotropy would likely cause us to overestimate the mean swimming speeds. Since the acoustic beams are oriented just off the horizontal axis and we rely on the higher sonar resolution in the range dimension, if the euphausiids were swimming mainly in the horizontal plane to maintain themselves at a constant depth, our emphasis on the horizontal swimming velocities would cause us to overestimate (not underestimate) the true swimming speeds.

Another possible source of error arises if currents are not constant during the time intervals over which the displacement distribution functions are calculated. Rapidly fluctuating currents would cause peak widths to broaden with increasing time delays, which would also lead us to overestimate the behavioral component of swimming velocities to some extent. Hence, any bias introduced by this factor would also lead us to report maximal daytime swimming velocities.

Our assumption that the swimming movements of euphausiids are uncorrelated implies that there is no significant schooling behavior. While *Euphausia superba* in the Antarctic is well known to form coherent schools (e.g., Hamner et al. 1989) and *E. pacifica* has been reported to form groups with parallel orientation at the sea surface in the Sea of Japan (Komaki 1967; Hanamura et al. 1984), we are not aware of any reports of daytime schooling behavior of *E. pacifica* in the vicinity of Saanich Inlet. Mackie and Mills (1983) observed dense aggregations of up to 10 000 animals·m^{-3} in very thin vertical layers above the oxycline in Saanich Inlet but made no remarks suggesting that these animals were arranged in schools with similar orientation and coordinated swimming motions.

Various other aspects related to the data processing and estimation merit discussion. Distances between the 20 strongest targets in each of the successive frames were used because this increases the likelihood of tracking the same set of targets from frame to frame. If the 20 strongest targets are even moderately uniformly distributed within the sonar volume, the closest target to a given target between frames should be the very same target. Figure 1 illustrates that the sonar records contain the reflections from many targets that are strong and can be followed from frame to frame. In addition, inspection of the target lists showed that there were many targets that were clearly identifiable and either drifting or moving very slowly. At longer frame delays (4 s was the maximum delay used) the possibility of mistaking target trajectories becomes more probable unless a specific target-tracking algorithm is use. As applied, our procedure provided reasonable estimates for both the current velocity and the spreading of the animal displacement distribution functions.

Consideration of the bias produced by requiring the animal to stay in the same beam is also an important question. Since the target identification procedure only recognizes targets that have stayed in the same beam, it is possible that the faster moving targets could be missed. Targets that are transiting from one beam to another will also be missed. In order to judge the effects of this potential source of bias, a computer simulation was performed. The simulation placed a set of targets inside a volume with the approximate dimension of one of the insonfied beams. Next, the targets were translated with a displacement that was the sum of a mean component plus a random component. The random component was an isotropic and normally distributed displacement function, designed to approximate the movement of the animals. The mean component was a uniform translation that was designed to approximate the effect of current. The relative angles between the beam and current were chosen so that the beam corresponded to the most downward pointing one. This beam would induce the maximum amount of narrowing of the displacement distribution function, as compared with the other beams, since the targets spend the least amount of time in it. The mean component was chosen to simulate a current of 7 cm·s^{-1} (the maximum current observed in the first year) and the random component was drawn from a normally distributed probability density function for velocity that was isotropic in angle with standard deviations of 0.25, 0.5, 1, 2, and 3 cm·s^{-1}. The total displacements were chosen to correspond to a tracking time of 2 s, identical to the tracking time for the experiments performed in the first year. The simulations indicated that a 10% narrowing of the displacement distribution functions should be expected. Since this beam is a worst case, we conclude that the bias introduced by the finite residence time of the particles in a single beam, with the faster moving particles "jumping" out, is of only minor significance.

The simulations also explain why, in Fig. 4, the displacement distribution functions from beam set 8 are somewhat wider than those of beam set 1, with an increasing discrepancy with increasing delay time. The targets in beam set 8 have a longer residence time because the current flow is mainly horizontal and this beam is more horizontally oriented than the rest. The longer residence time leads to a somewhat wider distribution function, as evidenced in the computer simulations, as the particles that are more active do not jump out of the beam with the same frequency as the particles that are in the beams that are pointing more vertically.

Studies of the in situ behavior of zooplankton have been constrained by the limited number of tools available (see Sprules et al. 1992). Many approaches introduce observer effects through intrusive lighting or hydrodynamic disturbances. Although direct SCUBA observations (e.g., Hamner 1984) have been invaluable, they are limited to a very shallow sector of the water column. The present results illustrate the utility of tracking sonar systems, especially when combined with optical verification of the identity of targets (Jaffe et al. 1998), for investigating the behavior of zooplankton in their natural environment. This permitted us to measure, for the first time, the quiescent swimming behavior of euphau-

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siids in daytime strata. Reduced motility confers simultaneous advantages of reduced metabolic costs and minimization of encounter rates with predators.

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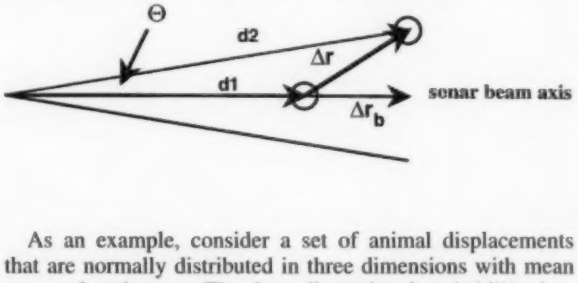
Appendix

An animal displacement of Δr in a single sonar beam is illustrated in Fig. A1. It is necessary to show that the "projection" of this vector onto the axis of the beam Δr_b is what is measured by the sonar. Existing methods to reconstruct functions from their projections can then be invoked (Kak and Slaney 1987). The animal's initial position is $d\bar{1}$, and after a short time, the animal is at position $d\bar{2}$, where $d\bar{1}$ and $d\bar{2}$ are vectors and the transducer is located at the origin of the coordinate system. Since in this analysis, we measure only range, the displacement measured by the sonar is $|d\bar{2}| - |d\bar{1}|$. However, since the vector $\Delta r_b = d\bar{2} \cos \Theta - d\bar{1}$, this is approximately equal to $|d\bar{2}| - |d\bar{1}|$, since these vectors are colinear and $\cos \Theta$ is approximately equal to 1 (0.9998) for a maximum angle of 1°, which is the half-angle that the beam subtends. Thus, at a range of 10 m, the maximum discrepancy that is possible with this approximation is 2 mm, far below the range resolution of the system.

Fig. A1. Geometric projection of the true three-dimensional displacement onto the range axis of the sonar beam.



As an example, consider a set of animal displacements that are normally distributed in three dimensions with mean zero and variance σ. The three-dimensional probability density function of the displacements can be represented as

$$(A1) \quad f(\Delta x, \Delta y, \Delta z) = \frac{1}{2\pi\sigma^2} e^{-\frac{\Delta x^2 + \Delta y^2 + \Delta z^2}{2\sigma^2}}.$$

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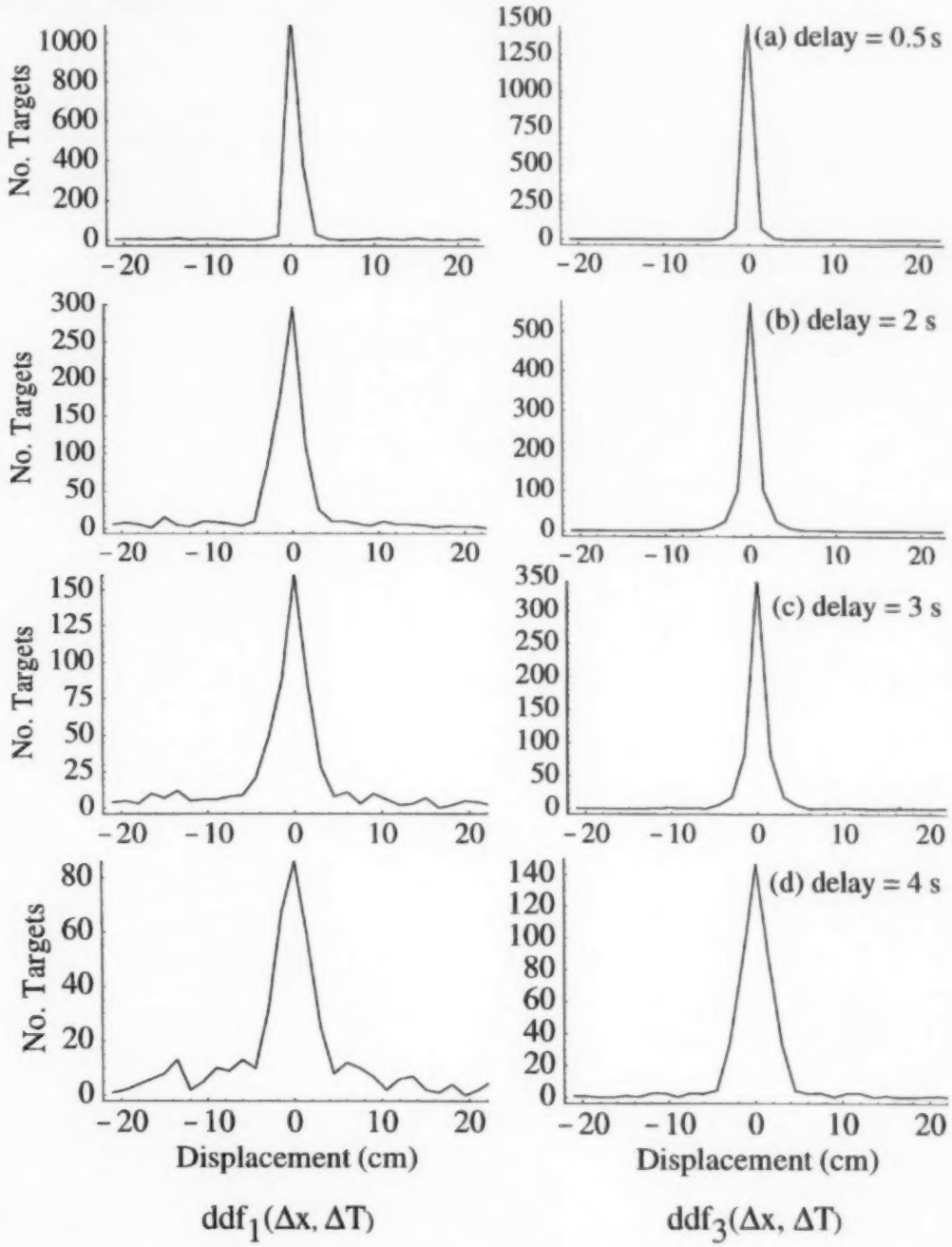
Fig. A2. Illustration of a set of displacement distribution functions before and after processing that allow the computation of $ddf_3(\Delta x,\Delta T)$ from $ddf_1(\Delta x,\Delta T)$.



In this case, the projection of this function onto a one-dimensional axis (here taken to be the *y*-axis with no loss of generality, since this function is isotropic) is the marginal probability distribution function

$$f(\Delta y) = \int\int \frac{1}{2\pi\sigma^2} e^{-\frac{\Delta x^2+\Delta y^2+\Delta z^2}{2\sigma^2}} dx dz \quad \text{(A2)}$$

which is equivalent to

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$$\text{(A3)} \quad f(\Delta y) = \frac{1}{2\pi\sigma^2} e^{-\frac{\Delta y^2}{2\sigma^2}}.$$

Evidently, since the projection of this normally distributed function onto a single axis results in a normally distributed function with equal variance, there is no need to invert for the "true" three-dimensional function.

Now, since the displacement distribution function is a sum of projected functions, via the linearity of the projection operator, it can be shown that the measured one-dimensional displacement distribution is a projection of the true three-dimensional displacement distribution function. Thus, inversion of the measured one-dimensional displacement distribution is what is desired. We present without proof here the necessary algorithm and refer the interested reader to the tomography literature (Kak and Slaney 1987) for details.

Given the one-dimensional displacement distribution function $ddf_1(\Delta y,\Delta T)$, the inversion can be accomplished via a one-dimensional Fourier transform $\mathcal{F}$ followed by a three-dimensional inverse Hankel transform $\mathcal{H}^{-1}$:

$$\text{(A4)} \quad ddf_3(\Delta y, \Delta T) = \mathcal{H}^{-1}\{\mathcal{F}[ddf_3(\Delta y, \Delta T)]\}$$

In order to implement this scheme, a computer algorithm was written in Mathematica (Wolfram, Ill.). The performance of the algorithm was verified for several sets of test data (normally distributed functions). Figure A2 demonstrates a set of displacement distribution functions before and after transformation. Some narrowing of the functions can be noted after transformation, which indicates that the functions are approximately but not strictly normal distributions.

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Skipper skill and panel data in fishing industries

Dale Squires and James Kirkley

Abstract: Skipper skill or managerial ability plays a central role in the harvesting of fish and fishing power. Examining the influences of managerial ability on catch rates, however, may be complicated, since managerial ability is generally unobservable. Using panel data on production activities in the Pacific Coast trawl fishery, we examine the use of the fixed- and random-effects panel data models to depict managerial skill by intervessel differences, representing differences in technical efficiency. The random-effects production model is selected over the fixed-effects model. We conclude that skipper skill is more related to finding fish, dealing with unforseen events, and handling inclement weather than it is to managing the economic inputs.

Résumé : L'habilité et les capacités de gestionnaire du capitaine de pêche sont des paramètres essentiels de la récolte et de la puissance de pêche. L'examen des effets de la capacité de gestionnaire sur le taux de capture peut cependant être compliqué car celle-ci est généralement impossible à observer. Nous examinons, à partir de données de panels sur la pêche au chalut de la côte du Pacifique, l'utilisation de modèles de données de panels à effets fixes et aléatoires pour décrire les capacités des gestionnaires à partir des écarts entre les bateaux, qui représentent des écarts d'efficacité technique. Le modèle de production à effet aléatoire a été retenu. Nous concluons que les habilités du capitaine de pêche ont plus trait à la capacité de trouver le poisson et de gérer les événements imprévus et le mauvais temps que de gérer les intrants économiques.

[Traduit par la Rédaction]

Introduction

Skipper skill or managerial ability is widely recognized as playing a central role in the harvesting of fish. Managerial ability is generally considered complex and traditionally has posed a considerable challenge to specifications of production or harvesting technologies. Griliches (1957) showed that omitting firm (vessel) management biases coefficients in production functions.

The notion that some captains of fishing vessels are more skilled in finding and catching fish and consistently enjoy higher incomes and net returns is widely recognized in the fisheries literature. In summarizing the anthropological literature, Thorlindsson (1988) observed that there are two notions of the skipper effect. First, numerous authors have argued that any skipper effect that may exist should be consistent, giving a relatively stable comparative ranking of fishing success across time. Hence, captains performing well in one season should perform well in the following season. Second, the other notion of the skipper effect is based on the performance of individual skippers. This second notion does not require a stable hierarchy of fishing success, but rather only a few noteworthy skippers with the catch more or less randomly distributed between the rest of the captains. In a dissenting view, Palsson and Durrenberger (1982) argued that other factors, such as vessel size and time spent fishing, are instead important.

The question arises when analyzing the fishery production process of how to specify consistent unobserved managerial differences between vessels. In this paper, we address the issue of managerial ability in the fishery harvesting technology and determinants of fishing power. The concepts of "skipper skill" or "the good captain hypothesis" are terms widely used to this denote managerial ability.

The paper is organized as follows. The second section reviews the concept and specification of unobserved managerial ability in production functions, where a production function gives the maximum flow of outputs in physical terms from quantity flows of inputs. The section then reviews how managerial ability has been considered in empirical fisheries studies. The third section, exploiting results from the econometrics of panel data, suggests an approach that has yet to be applied to fisheries. Panel data are pooled cross-section and time series data, giving multiple observations on each individual in the sample, and are sometimes called longitudinal or temporal cross-sectional data. The panel data approach with fishery production functions explicitly accounts for consistent heterogeneity across vessels and equates these differences with variations in technical efficiency. The fourth section provides an empirical application and some policy implications.

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Materials and methods

Literature review

Management has been introduced into the production function in several different ways. One approach specifies entrepreneurship or management as a fixed input (an input that does not change its level) for the individual firm, dividing the managerial function into

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"uncertainty bearing" and "supervision and coordination." In another approach, Mundlak (1961) and Hoch (1962) introduced management as a residual factor through the use of firm-specific dummy variables. The Mundlak and Hoch approach is now referred to as the fixed-effects approach to panel data. The fixed-effects approach assumes that whatever management is, it does not change considerably over time,and that for short periods of several years, it can be assumed constant (Mundlak 1961).

The fixed-effects approach and increasing availability of panel data have made it possible to assert that major mispecifications of the production function, which are transmitted to input decisions, such as differences in resource abundance, management, and labor quality, are largely fixed over time (or at least over the length of the available panel) and hence can be eliminated by an appropriate "within" transformation (Griliches and Mairesse 1997).

Mundlak (1961, p. 340) observed that since management is introduced as a residual variable, there is a danger that in what we refer to as management, we also include the effects of factors that do not depend on the management but rather on the particular environmental conditions of the firm. In that case, it will be an estimate of the management and these environmental effects combined. A portion of the environmental effect can be removed by grouping the firms into homogenous groups where management is randomly distributed over these groupings (Mundlak 1961).

The problem of picking up the effect of other factors analogous to the firm effect raised by Mundlak (1961) may not be as serious in some fisheries as in agriculture or some other industries, depending on the mobility of capital (fishing vessels) and fish stocks. To the extent that fishing vessels harvest in different areas during the year, then environmental and resource effects of any given year may not entangle themselves with the management effect. This smoothing out of the spatial and temporal differences in resource abundance and density, weather conditions, ocean currents, topography of the ocean floor, migratory patterns of some fish, and other factors will vary by fishery and depends on territoriality and other factors contributing to immobility of vessels. For example, to the extent that demersal fish are reasonably sedentary, or at least spatially clumped, fishers may develop knowledge of their behavior and location. Fishers may return to the same grounds to harvest, in which case the area or farm effect could be of some importance. For example, fishers in the lobster fishery of Maine developed de facto territorial and use rights to harvest the same area (Acheson 1975, 1981), contributing to an area effect. Hilborn and Ledbetter (1985) found a definite area effect due to characteristics of the fishery.

The approach of Mundlak (1961) also assumed that managerial skills cause neutral shifts in the production function with no change in the slope of the production function or relationship between individual inputs and output (technically, production elasticities). There is no reason to expect management to affect all inputs equally and neutrally. To the extent that better managers adopt new technology sooner, a confounding of management and technology occurs. Along similar lines, Yang (1997) observed that education affects not only managerial efficiency, but also worker productivity, so that the effects of education should be accounted for in both management and labor (such as quality-adjusted labor units). Yang (1997) further observed that in an environment of imperfect information, education affects managerial productivity through the skills of obtaining and using information for managerial decisions, including allocation of inputs and choice of products to produce. Uncertainty-bearing is also included in the management dummy variables.

Other researchers used proxy variables for the age, education, and experience of managers or principal components analysis of personal characteristics of managers to derive a proxy variable for management. Holt (1982) used a measure of fishing skill, based on the proportion of successful pursuits adjusted for vessel characteristics and days of effort, designed to distinguish between professional and nonprofessional vessels. As with all proxy variables used to capture something with no observable counterpart, measurement error and bias can follow, although the asymptotic bias expected from inclusion is generally smaller than from exclusion. Using a Cobb–Douglas functional form for the production function, Muller (1974) allowed the coefficients of physical inputs to depend on characteristics related to differences in management quality. (A functional form is the particular mathematical form of the production function.)

Another approach to measure and incorporate management into the production function employs performance rankings or indexes of each production unit compared with the other production units (Campbell 1991). Several limitations can arise when using indexes of management derived from performance rankings. Mundlak (1961, p. 336) warned that an attempt to substitute some index of management does not solve the conceptual difficulty, and that this attempt can be regarded as an ad hoc procedure as long as no criterion for evaluating its performance is available. More concretely, Mefford (1986) observed that simultaneity bias may exist between any performance-based management measure and output. Mefford (1986) suggested lagging the management variable by one time period as a possible solution (although this would necessarily require at least two periods of data on the same or most of the same firms or vessels, i.e., panel data).

Skipper skill is related to information gathering and utilization, including finding and catching fish, knowing or sensing when to leave a spot for another, managing and supervising crew, responding to breakdowns in equipment and machinery, responding to changing tides and weather, seasonal variations in resource abundance, and numerous other factors. By the time a captain has decided when to make a fishing trip, the crew size, gear type, and likely fishing areas may have already been decided. Thus, the captain's options for changing the levels of inputs can be severely limited. The skipper must make do with the available resources and be able to readily change in response to changing circumstances (e.g., finding a "hot spot" or large concentration of high-valued fish). The skipper must regularly inspect the gear after nearly every "haul-back" to prevent unnecessary gear damage and the subsequent loss of fish. All in all, in this hunting production process, it appears that skipper skill or managerial ability is more indicative of the ability of a vessel captain to find fish and to readily change operations in response to unforeseen changing circumstances rather than make changes in input levels.

Barth (1966) stressed that the decision on where to search for herring in the Norwegian herring fishery is the first and most important decision affecting the size of the catch. Acheson (1981) identified three components of skipper skill: (*i*) the ability to accurately navigate to find the best grounds, (*ii*) good knowledge of the ocean, such as its currents, depths, and types of bottom, and (*iii*) good knowledge of the species of concern. Thorlindsson (1988) identified three additional components: (1) the ability to "read the sea" and its ecological environment, (2) the willingness of the skipper to search independently and to take calculated risks, which is probably a function of many factors, and (3), the ability of a skipper to lead and manage the crew.

Skipper skill may also be related to minimizing risk and uncertainty. Palsson and Durenberger (1982) suggested that skippers avoid seeking high catches with low probabilities and instead seek high probabilities of some catch, even if a small one. Skipper skill has been equated to technical efficiency (technical efficiency occurs when the maximum amount of an output is produced for a given set of economic inputs, given the resource stock and state of technology) by Kirkley et al. (1998).

In empirical studies, Palsson and Durrenberger (1982) found no relationship between skipper experience and catch rates. Acheson (1975), reporting the results from a regression analysis, indicated that skipper age was not a good surrogate variable for fishing skill and that education was marginally statistically insignificant.

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Squires et al. (1998) found that skipper characteristics were generally statistically insignificant in Peninsular Malaysian gillnet fisheries as variables explaining technical inefficiency.

Panel data approach to specifying managerial ability

Panel data techniques account for heterogeneity across production units and allow control for omitted variables that are persistent over time (Hausman and Taylor 1981). Panel data sets are more oriented towards cross-section than time series analyses — they are "wide but short." The panel data approach can be employed when the number of cross-sectional units is large and the number of time periods over which those units are observed is small. Heterogeneity across units is often the central focus of the analysis, but the inclusion of time series of cross-sectional units allows dynamics or stochastic processes to be considered. The estimated coefficients from a single cross section are more likely to reflect interfirm or intervessel differences than intrafirm or intravessel dynamics, the more so that the panel data are more fundamentally cross-sectional than time series — "wider rather than deeper." If a firm or vessel's harvesting technology remains constant or changes slowly or if changes are explicitly controlled for, panel data allow evaluation of before-and-after effects, say from regulations (Hsiao 1986). The increased numbers of observations from additional cross sections provide a number of statistical improvements: they increase the efficiency of parameter estimates, reduce the problem of multicollinearity by augmenting degrees of freedom and information on individual attributes, help the identification of economic models and discrimination among competing economic hypotheses, and eliminate or reduce estimation bias with improved specifications (Hsiao 1986).

The fixed-effects panel data approach to incorporate unobserved vessel-level managerial differences in production functions, introduced by Hoch (1962) and Mundlak (1961), assumes that differences across units can be captured by differences in the constant term, that management remains equally effective over time for the individual production unit, such as a vessel, and that differences between units are due to managerial differences and not some other unit-specific factor. This generalization of the constant intercept and slope model for panel data introduces dummy variables to account for the effects of those omitted variables that are specific to individual cross-sectional units but stay constant over time. This one-way fixed-effects model is also called the analysis of covariance or least squares dummy variable model; a recent summary is provided in Balestra (1996). Under a fixed-effects approach, the results are conditional on the unobserved fixed effects, and their distribution plays no role (Chamberlin 1984). The one-way fixed-effects model accounts for intervessel differences such as managerial ability, i.e., skipper skill. In the following empirical study, given the relatively short time period covered by our analysis, 1986–1989, it is plausible to assume that relative skipper skill, equated to intervessel differences, remains stable.

Effects that are specific to each time period but are the same for all cross-sectional units (i.e., time-specific effects) give a two-way fixed-effects model when added to the one-way fixed-effects model. In this study, we use annual dummy variables for 1986, 1987, and 1988 to account for these time-specific effects that primarily reflect changes in resource abundance and to a lesser extent (given the short time period) changes in the state of harvesting technology. Dummy variables are used given the interpretation of resource abundance as a technological constraint that shifts the production technology.

The random-effects (variance components) model may also be used to examine unobserved managerial differences among production units, such as vessels or firms (Chamberlin and Griliches 1975). The approach has also been used to examine unobserved factors such as individual ability in the returns to education (Chamberlin and Griliches 1975); a recent summary is provided in Matyas (1996). The random-effects approach specifies an overall intercept and an error term (V_{it}) with two components: ε_{it} and μ_i. The ε_{it} is the traditional error term unique to each observation. The μ_i is an error term representing the extent to which the intercept of the ith cross-sectional unit differs from the overall intercept. The vessel-specific intercepts, μ_i, are treated in the same manner as the traditional error term, ε_{it}, except that the intercepts constitute the time-invariant component $E[\mu_i] = 0$, $E[\mu_i^2] = \sigma_\mu^2$, $E[\mu_i \mu_j] = 0$ for $i \neq j$ and $E[\mu_i \varepsilon_{it}] = 0$. All disturbances have variance $E[V_{it}^2] = \mathrm{Var}[\varepsilon_{it} + \mu_i] = \sigma^2 = \sigma_\varepsilon^2 + \sigma_\mu^2$. A third error term could be added for time-specific effects, but fixed time-specific effects are assumed for the biomass and state of technology rather than treating it as a random variable.

The choice of a fixed- or random-effects model depends on the context of the data, application of the results, and statistical tests. If the data exhaust the population, then the fixed-effects approach, which produces results conditional on the units in the data set, is reasonable. Differences between units can be viewed as parametric shifts of the production technology and are particularly applicable when inferences are confined to the effects of the model. When inferences are made about a population of effects from which those in the data are viewed as a random sample, the effects should be viewed as random. Thus, individual specific constant terms are viewed as randomly distributed across cross-sectional units. Mundlak (1978) argued that individual effects are always random and that fixed-effects models simply analyze conditionally on the effects present in the observed sample. The random-effects model also conserves degrees of freedom. The random-effects model presupposes that the specific behavior of the observed pooled units and the different time periods are not really well known (Matyas 1996). Nerlove and Balestra (1996) suggested that the fixed-effects models may be appropriate when the population is sampled exhaustively or when it is desired to predict individual behavior (such as the probability that a given individual in a sample will do something) but that random-effects models are more consistent with the view that the "population" modeled in econometrics consists not of an infinity of individuals but of an infinity of decisions.

The random-effects model assumes that the random error associated with each cross-sectional unit α_i is uncorrelated with the other explanatory variables, which leads to omitted variable bias for the other explanatory variables if incorrect (Mundlak 1978). Orthogonality between the random effects and the other regressors can be tested by the specification test devised by Hausman (1978) and Hausman and Taylor (1981). This χ^2 specification test is used to test for the fixed-effects model versus the random-effects model, where large values argue in favor of the fixed-effects model over the random-effects model. Breusch and Pagan's (1980) Lagrange multiplier statistic is used to test the random coefficients model against a classical model of regressors without fixed effects, where large values argue in favor of a fixed-effects model. F tests are also used to test fixed effects against the classical regression model without fixed effects. (The Lagrange multiplier test statistic is distributed asymptotically as χ^2 with degrees of freedom equal to the number of independent restrictions being tested. (Godfrey 1988).) Details of these tests are given in Godfrey (1988) and Hsiao (1986).

Differences in the intercepts across firms or vessels, whether from a fixed- or random-effects model, reflect technical inefficiency, since they represent failures to achieve maximum possible output (catch) given the inputs (Cornwell and Schmidt 1996). Thus, managerial differences or skipper skill, to the extent that they are represented by intervessel differences in catch rates for given bundles of inputs, are fundamentally differences in technical efficiency, as argued by Kirkley et al. (1998). Similarly, intervessel differences in profits (as opposed to catches) correspond to differences in not only technical efficiency, but also scale efficiency (the profit-maximizing level of production) and allocative efficiency (the profit-maximizing mix of inputs given their prices).

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U.S. Pacific Coast groundfish trawl fishery

The panel data approach to specifying and analyzing unobserved differences between vessels is applied to a study of the Pacific Coast groundfish trawl fishery. We assume that these unobserved differences are due to variations in management, i.e., skipper skill. We further assume that captains remain on the same vessel over the 4 years of the study, 1986–1989. Attrition of vessels from the panel did not pose a problem.

The groundfish fishery on the Pacific Coast of the United States is one of the most important commercial fisheries occurring off California, Washington, and Oregon. It provided between 20 and 30% of revenues from all Pacific Coast fisheries in 1987 and 1988. This fishery is also the most diverse fishery on the Pacific Coast. The commercial groundfish fishery is comprised of several different gear and vessel combinations, which are used to harvest a wide variety of species. These gear types include groundfish trawls (bottom and midwater), fish pots and traps, setlines and longlines, gill nets, set nets, and other miscellaneous gears. The major species harvested include Dover sole (*Microstomus pacificus*) and other flatfishes, sablefish (*Anoplopoma fimbria*), shortspine (*Sebastolobus alascanus*) and longspine thornyhead (*Sebastolobus altivelis*), widow rockfish (*Sebastes entomelas*), other rockfish, Pacific cod (*Gadus macrocephalus*), Pacific hake (*Merluccius productus*), and pink shrimp (*Pandalus jordani*).

The trawl fleet is the most important sector of the groundfish fleet. Vessels in the fleet range from 10 to 34 m in length, with hull volumes generally under 200 gross registered tons. Engine power ranges from 100 to 1000 hp. The average crew size is three for most vessels and around four for catcher vessels targeting Pacific hake with midwater trawls. Only a small number of firms operate multiple vessels, so that most vessels are owner-operated, and there is very little vertical integration between the fishing vessels and onshore processors. The average annual number of vessels fishing in the groundfish fishery was 374 between 1981 and 1989.

Harvesting technology

The fishing trip forms the basic time unit. A fishing trip generally lasts anywhere from 1 to 5 days and begins with a fueled, stocked vessel leaving port. Based on information about weather and ocean conditions, the expected abundance of species, and their expected relative prices, fishers make decisions about where and when to fish. Once at the site, fishers target those species believed to generate the highest profit by adjusting nets, dragging depth, towing speed, and other factors. Vessels can also change fishing grounds, type of bottom fished, and net types during a trip; these changes typically alter the species composition and sometimes size of the harvested fish and pink shrimp. Smaller vessels tend to make more frequent, shorter trips, while larger vessels tend to make longer but fewer trips in a week.

Trip lengths vary depending on the fishing location along the Pacific Coast. The continental shelf is narrower in the south and wider in the north. The length of a trip tends to increase from south to north, but the frequency of trips correspondingly tends to decrease in this direction.

The production function models the production process as the application of economic inputs to the fish stock to yield a flow of catch. The annual stock-flow production technology can be specified as

$$(1) \qquad Y = f(K,L,F;B,T)$$

where Y represents output (catch) in metric tons, K denotes capital, L is labor, F is fuel consumption, B represents biomass of the resource stock, and T denotes technological factors such as the state of technology. We aggregate the different species into a single aggregate output (catch) by a weighted average, where revenue shares form the weights.

Capital is a multidimensional concept and includes engine horsepower, net type, mesh, vessel size, vessel electronics, and other gear and equipment (e.g., winches). A single index of capital does not exist, and thus, boat length (registered length overall in metres) gives a proxy variable for the heterogeneous capital stock. The other dimensions of capital are closely related to length, and length (as opposed to a measure of vessel volume such as gross or net registered tons) is the measure most commonly used by fishers and by which vessels are sold. Fleet size was stable over the period of analysis, so the stock of capital is assumed to be in full static equilibrium, giving a flow of capital services directly proportional to the capital stock.

Labor is represented in the model by the flow of labor services and measured as the product of crew size (including captain) and by weeks fished in a year. Fuel consumption is represented by litres of fuel consumed per year. Resource stock is interpreted as a technology shift variable in the stock-flow production technology, i.e., a technological constraint, rather than as an input under the control of individual firms. Hence, it is represented by annual dummy variables (Squires 1992; Kirkley and Strand 1988).

Data

The analysis covers 1986–1989. The panel data set encompasses annual costs, revenues, landings, and fishing time on 83 vessel-years from 26 trawl vessels that participated in the Pacific Coast groundfish fishery in Washington, Oregon, and northern and central California. The sample tends to evenly represent vessels from northern California, Oregon, and Washington, with only a few from central California. The data set has not been used before in a production model, although some of the data have been used in the calculation of multifactor productivity indices (Squires 1992). The data form an unbalanced panel data set, since the number of time series observations is not always the same for each cross section.

The data were obtained from the National Marine Fisheries Service's West Coast Cost-Earnings Data Base (Herrick et al. 1992). The cost data were originally obtained from confidential income tax files of the National Marine Fisheries Service's Capital Construction Fund program and Fishing Vessel Obligation Guarantee loan program. Given the source of the cost information, the data are not randomly drawn from the population and are likely subject to selection bias toward more successful vessels. Hence, the empirical results can only be viewed as indicative or suggestive of the entire fleet and illustrative of the panel data approach, although the more successful vessels do tend to harvest a greater proportion of the total harvest and are frequently of special concern to regulators.

Annual fuel consumption data were computed by dividing the annual fuel expense of each vessel by the average annual unit fuel price that was observed at the port closest to the port of principle landings of the vessels. Fuel expense data came from federal income tax forms, and fuel prices were obtained through a quadrennial telephone survey of 32 marine fuel docks coastwide on the cash price of 2200 L of No. 2 marine diesel fuel. The fuel cost data were only available in annual form. Hence, the analysis was conducted on an annual basis, with all variables defined in terms of years, even though the trip forms the most fundamental time unit. Vessel length data were obtained from the U.S. Coast Guard. Landings and revenue information originally came from the PacFIN database of the National Marine Fisheries Service, which in turn originally came from landings receipts filled out by fish processors when fishers landed their catch. All revenue and cost data are in constant 1989 U.S. dollars, where inflation was corrected for by the GNP implicit price deflator. Table 1 summarizes the salient features of the data set used in the model.

Hypothesis tests and final form of the technology and model

Given that different functional forms of the production function impose different restrictions on the underlying harvesting technol-

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Table 1. Descriptive statistics of the data for 26 Pacific Coast groundfish trawlers, 1986–1989.

Variable	Mean	SD	Minimum	Maximum
Gross registered tons	74.39	40.64	10	169
Length (ft)	59.33	14.36	28	86
Crew size (persons)	3.01	0.348	2.0	4.0
Weeks fished	30.46	7.57	9.0	44.0
Fuel cost (1989 $US)	15 181	18 333	4680	97 000
Total catch (metric tons)	559.55	900.3	2.2	5110
Total revenue (1989 $US)	36 975	53 452	1.8	204 500

Note: Arithmetic sample means of annual data for 26 vessels and 83 total vessel-years.

ogy, it is necessary to statistically examine for different possible functional forms of the technology. We can consider three different functional forms for the production function, starting with the most general and progressing to the most restrictive: translog, constant elasticity of substitution (CES), and Cobb–Douglas. We also consider the Cobb–Douglas with variable and constant returns to scale. (Returns to scale measures the proportional increase in output for a 1% increase in all inputs.) The various functional forms and the returns to scale are easily tested by imposing the parametric restrictions consistent with each hypothesis and tested via nested hypothesis testing. We commence the testing with the most general and least restrictive and progress towards the least general and most restrictive functional form.

Our most general functional form for the production function is the translog. We next consider the CES production function. We last consider the more restrictive Cobb–Douglas production function. The translog functional form allows variable elasticities of substitution (elasticities of substitution measure the vessel's capability to change the mix of inputs). The CES imposes a constant elasticity of substitution among all inputs, although the value is empirically determined. The Cobb–Douglas technology implies constant and unitary elasticities of substitution between all inputs. The CES and Cobb–Douglas both impose homotheticity (input usage remains in fixed proportions for any output level) and global separability among inputs (the ratio of first partial derivatives of output with respect to an input for two any inputs — the marginal product — is invariant to changes in the level of any other, third input).

Starting with the translog, the hypothesis tests for functional form follow this sequence: (*i*) translog versus the null hypothesis of CES (the CES restrictions are imposed on the more general translog), (*ii*) CES versus the null hypothesis of Cobb–Douglas (the Cobb–Douglas restrictions are imposed on the more general CES), and (*iii*) given the Cobb–Douglas, constant returns to scale versus the null hypothesis of variable returns to scale. Each ensuing hypothesis is tested given that the previous one holds. The testing stops whenever a null hypothesis is rejected.

The statistical significance of area dummy variables is also tested. Area dummy variables allow for spatial differences in resource availability according to home port in four regions on the west coast: Washington, Oregon, northern California, and central California. Home port was assigned by the plurality of revenues for each year (home port can vary annually). If vessels travel substantial distances to fishing grounds, the area dummy variables may not fully capture the spatial differences in resource availability. For this case, the home port may not serve as a good proxy variable for spatial differences in resource availability. While a better measure may be to assign the spatial variable based on the plurality of time spent in different fishing grounds, these measures are not available in the PacFIN database. This issue is likely to be more important for annual as opposed to trip data.

The overall testing procedure is not fully nested because more general models do not fully encompass or nest more specific tests. The tests for the classical regression model versus fixed effects versus random effects and the test for the area dummy variables are not part of the strict nested testing sequence for functional form. The overall significance of the hypothesis tests may be, as Godfrey (1988) noted, somewhat smaller than the upper bound of S_α, where α is the significance level for each individual test and S is the number of tests. The power of these tests cannot be assessed because we do not have adequate data, particularly on fuel consumption, to assess the underlying population required to assess sample selection and hence power.

The translog production function, which we initially consider, may be written as

$$(2)\qquad \ln Y = \alpha + \Sigma_a \beta\, D_A + \Sigma_b \phi_b D_B + \Sigma_i \alpha_i \ln X_i + 1/2 \Sigma_i \Sigma_j \alpha_{ij} \ln X_i \ln X_j$$

where X_i and X_j represent the economic inputs (capital services, labor services, fuel consumption), D_A represents dummy variables for areas, and D_B represents dummy variables for year. Symmetry is imposed in eq. 2 so that $\alpha_{ij} = \alpha_{ji}$.

The translog production function was interpreted as a second-order Taylor's series approximation to an unknown, underlying function. All data were scaled by the geometric mean so that the point of approximation was the geometric mean of the sample. In addition, at this point, the translog, interpreted as a second-order approximation, exactly coincides with the translog interpreted as the true functional form (Boisvert 1982).

The (homogeneous) translog production function is a quadratic approximation to an arbitrary CES function when specified as follows (Kmenta 1964):

$$(3)\qquad \ln Y = \alpha + \Sigma_a \beta\, D_A + \Sigma_b \phi_b D_B + \Sigma \alpha_i \ln X_i + \Sigma \Sigma \alpha_{ij} (\ln X_i - \ln X_j)^2,\ i \neq j.$$

Rather than directly test the appropriate restrictions on the translog (cf. Field 1988), software restrictions required separately estimating the translog and linear approximations to the CES, eq. 3, and testing via an F test.

The Cobb–Douglas in logarithmic form may be written as

$$(4)\qquad \ln Y = \alpha + \Sigma_a \beta\, D_A + \Sigma_b \phi_b D_B + \Sigma \alpha_i \ln X_i.$$

The Cobb–Douglas may be obtained from the translog or CES if $\alpha_{ij} = 0$, $\forall\ i$ and j, in eq. 2 or 3, respectively. The restriction for constant returns to scale with the Cobb–Douglas form is $\Sigma \alpha_i = 1$ (so that a 1% increase in all inputs yields a 1% increase in output, given the resource stock and state of technology). This restriction is directly imposed and tested by an F test. The procedures to determine the Cobb–Douglas versus the CES form require imposing the restrictions of $\alpha_{ij} = 0$, $\forall\ i$ and j, in eq. 3 and conducting an F test of the restrictions. If the null hypothesis of Cobb–Douglas is not rejected, homogeneity and constant unitary elasticities of substitution are maintained hypotheses. The test of the null hypothesis for constant returns to scale, conditional on the Cobb–Douglas technology, is an F test of $\Sigma \alpha_i = 1$ imposed on eq. 4.

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Table 2. Significance tests of model.

Test	Restrictions	Significance test statistic	Reject	Test type
Fixed-effects vs. classical model	25	10.163	No	F test
Random-effects vs. classical model	1	6.541	Yes	Lagrange multiplier
Fixed effects vs. random effects	15	21.691	No	Hausman's χ^2
No area vs. three area dummy variables	3	0.1667	No	F test
Translog vs. CES	3	0.548	No	F test
CES vs. Cobb–Douglas	3	0.482	No	F test
Constant vs. variable returns to scale Cobb–Douglas	1	14.195	Yes	Wald's χ^2

Note: Tests proceed in the order listed. Null hypothesis presented second for each test. A 0.01 level of significance for each test and a maximum of 0.07 level of overall significance.

Quantitative differences in skipper skill, interpreted as differences in technical efficiency, can readily be obtained by normalizing on the largest fixed or random effect (Cornwell and Schmidt 1996). This step, however, extends beyond the scope of this paper.

Empirical results and discussion

Statistical estimation

Estimation of the production function was initially by ordinary least squares. Zellner et al. (1966) noted that when output is stochastic due to "acts of nature" such as found in fisheries, firms select input levels to maximize expected profit. These input selections are subject to "human errors," which are uncorrelated with the stochastic error term in the production function. Hence, ordinary least squares of the production function gives consistent parameter estimates (Campbell 1991).

The presence of first-order serial correlation, a strong possibility with annual time series data, was tested on the classical regression model (without panel data considerations) by the Durbin–Watson test. Given the test value of 1.17, all subsequent regressions, including fixed- and random-effects models, were corrected for first-order serial correlation for each vessel.

The number of significance tests for overall model and functional form selection was seven: (*i*) one for the F test of fixed effects for skipper skill versus classical regression, (*ii*) one for the Lagrange multiplier test for random effects versus classical regression, (*iii*) one for the Hausman χ^2 test for the random-effects model versus the fixed-effects model, (*iv*) one for the F test of the area dummy variables, (*v*) two for the nested F tests of the final form of the production function, and (*vi*) a nested Wald test for constant returns to scale given a Cobb–Douglas functional form. (The Wald test statistic is asymptotically distributed as χ^2 under the null hypothesis with degrees of freedom equal to the number of independent restrictions (Godfrey 1988).) With the individual significance of each test set at 0.01, the overall significance was bounded from above by 0.07. Because the annual dummy variable was retained throughout, the fixed-effects model is one-way for intervessel differences: skipper skill and technical efficiency.

The F test for fixed effects versus the null hypothesis of the classical regression model without group effects did not reject the null hypothesis, since the F test statistic was 10.163 and there were 25 df (equal to the 25 restrictions for the dummy variables for vessels; recall that there were 26 vessels in total) (Table 2). The Lagrange multiplier test for the random effects versus the null hypothesis of the classical regression model gave an Lagrange multiplier value of 6.541 (Table 2). With 1 df (corresponding to the restriction for the error term of μ_i in the random-effects model), the null hypothesis was rejected in favor of the random-effects model (Table 2). In Hausman's χ^2 test for fixed effects versus the null hypothesis of random effects, the χ^2 value of 21.691 with 15 df (equal to the number of regressors) argues in favor of the random-effects model. A large value of the Lagrange multiplier statistic in the presence of a small Hausman statistic favors the random-effects model (Greene 1995).

The null hypothesis of no area dummy variables was not rejected given the F test statistic and 3 df (the number of parametric restrictions for the three area dummy variables) (Table 2). The null hypothesis of the CES functional form, as opposed to the translog, was not rejected, given the F test statistic of 0.54 and 3 df (Table 2). Next, the null hypothesis of the Cobb–Douglas functional form was not rejected, as opposed to the CES, given the F test statistic of 0.48 and 3 df. Finally, the Wald χ^2 value of 14.20 with 1 df suggests rejection of the null hypothesis of constant returns to scale in favor of variable returns to scale for the Cobb–Douglas functional form.

In summary, results from the statistical tests indicated a random-effects Cobb–Douglas production function without area dummy variables and with variable returns to scale as the final form of the production technology (Table 2). Apparently the Pacific Coast can by treated as a single fishing ground by the groundfish trawlers in the sample (with annual data), due to movement along the coast to different fishing grounds over the year by the trawlers. Table 3 provides estimates and results based on the ordinary least squares, fixed-effects, and random-effects models for the initial translog and Cobb–Douglas specifications.

The issue of robust results arises. First, because the testing is not nested, it is possible that changing the order of the nonnested tests could give different results. For example, testing first for functional form and then the proper panel data specification could give different results. Second, the model specification is fairly complex and a hypothesis test procedure such as this one may represent overfitting when there are a limited number of observations such as in this paper. When the data are not from a randomly drawn sample, some selection bias undoubtedly exists. Heterogeneity across vessels is the central focus of this analysis, not intravessel dynamics or stochastic processes, so that the emphasis should be placed on expanding cross-sectional size and representativeness of the sample, and the intertemporal length is

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Table 3. Estimated parameters.

Variable	Translog full model			Cobb–Douglas random effects final model
	Ordinary least squares	Fixed effects	Random effects	
Constant	–0.1004 (0.3954)	0.1755 (0.3709)	0.0652 (0.4825)	–0.2370 (0.2903)
DWASH	0.3017 (0.4115)	1.0042 (0.5589)	0.1931 (0.6405)	
DOR	0.3143 (0.1608)	0.2032 (0.1825)	0.0170 (0.2592)	
DCCAL	–0.0435 (0.3437)	–0.5981 (0.3817)	–0.2269 (0.5290)	
D86	–0.8097 (0.5142)	–0.7302 (0.4100)	–0.2731 (0.5385)	–0.0321 (0.5144)
D87	–0.2923 (0.4658)	–0.0208 (0.3590)	0.3727 (0.4206)	0.3773 (0.3734)
D88	–0.1780 (0.4260)	–0.4500 (0.3298)	0.6541 (0.2968)	0.6590 (0.2963)
Labor	1.4226 (0.3896)	0.6939 (0.5848)	0.5181 (0.3789)	0.6626 (0.2949)
Fuel	0.1061 (0.1324)	0.0937 (0.1687)	0.2567 (0.1709)	0.2896 (0.1577)
Capital	1.7070 (0.8004)	1.5111 (0.9651)	2.1667 (1.1582)	2.8348 (0.8547)
Labor	0.4315 (0.6185)	1.2172 (0.9675)	0.1113 (0.5805)	
Fuel	0.1084 (0.0922)	0.1864 (0.1117)	–0.0168 (0.1072)	
Capital	1.5167 (2.6110)	–4.5013 (3.7630)	–2.9895 (4.2481)	
Labor–fuel	–0.0005 (0.2694)	1.4149 (0.4869)	0.2934 (0.2791)	
Labor–capital	–3.2694 (1.5251)	–8.5842 (2.3831)	–2.3069 (1.7810)	
Fuel–capital	–0.2467 (0.7380)	0.3239 (0.9717)	0.3221 (1.0152)	
R^2	0.76	0.99	0.61	0.58
Observations	57	57	57	57

Note: Ordinary least squares. Correction for first-order serial correlation. Standard errors in parentheses. Translog point of approximation at geometric mean. DWASH, DOR, and DCCAL are area dummy variables for Washington, Oregon, and central California. Vessel-specific dummy variables in fixed-effects translog model not reported to conserve space.

reasonable for analysis of intervessel heterogeneity. Ideally, the power of the tests and selection bias would be formally evaluated, so that these results might best be viewed as suggestive or preliminary in that regard. In addition, parameter, marginal product, and elasticity estimates may reflect a combination of incomplete data, multicollinearity between inputs, inflexible functional form, and poorly measured fundamental inputs. Third, outliers could potentially impact the regression results. For example, there is one vessel in the sample that caught 2 tons and apparently spent at least $4680 for fuel. A formal outlier analysis is required to assess the impact of this and other potential outliers. However, we are unable to do so due to the length between the actual estimation of the model and the writing of the paper, in which the data set were misplaced.

The marginal products (the first partial derivative of output with respect to an input), calculated at the geometric mean (as $\partial Y/\partial X_i = (\partial \ln Y/\partial \ln X_i)(X_i/Y) = \alpha_i(X_i/Y)$), are all positive, indicating that the vessels operate in the appropriate range of production and the condition of monotonicity is satisfied, i.e., that an increase in an input increases output. The marginal products are 0.82 for capital, 0.51 for fuel, and 0.21 for labor.

The adjusted R^2 of the ordinary least squares estimate of the translog production function prior to panel data estimation was 0.76, i.e., 76% of the variation was explained by within-vessel characteristics. Adding the dummy variables of the fixed-effects model raised the adjusted R^2 to 0.99. Hilborn and Ledbetter (1985) found a similar jump in explained variation when explicitly accounting for intervessel differences. Nonetheless, the majority of the variation in this study was from within-vessel differences rather than from differences across vessels, which may be due to the inclusion of fuel consumption and labor in the fishery production function, correction for serial correlation, specification of total revenue rather than total catch as the dependent variable, and explicit use of panel data models while controlling for vessel size, area, and time. The high mobility of Pacific Coast trawl vessels, multiple species caught, and use of annual data (which masks the different specialized fishing strategies over the year) may also contribute. In contrast, Hilborn and Ledbetter (1985), who examined intervessel differences in value of catch (total revenue), found that close to 24% of the variation was due to intervessel differences or the skipper effect. Thorlindsson (1988) found that the intervessel skipper effect explained 35–49% of the variation in catch. Acheson (1975) found that 39% of the total variation in expected gross income was unexplained. Palsson and Durenberger (1982) found that a skipper effect accounted for only very little unexplained variance of intervessel catch rates in Iceland. Finally, the first-order labor coefficients are smaller in the translog models when the effects of unobservable fishing skill are introduced by the panel data approach.

The selection of a random coefficients model over a fixed-effects model, and the results of the Hausman test in particular, suggest that the random intervessel differences are uncorrelated with the other explanatory variables of capital, labor, and fuel. This finding suggests that differences in vessel-level management and technical efficiency — managerial ability or skipper skill — are not directly correlated with a skipper's management of the economic inputs. Rather, as expected in many fisheries, skipper skill and technical efficiency are more directly related to finding fish, dealing with unforseen events, handling inclement weather, and so forth.

In summary, the finding of consistent intervessel differences over the 4 years of panel data, after controlling for vessel size, variable input use, area, fishing time, annual differences over time, functional form, and serial correlation, provides supporting evidence to the notion of skipper skill as

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technical efficiency related to differences in catch rates that persist over time. To refine this panel data approach, subsequent research, when the data are available, might control for additional measurable variables that vary between vessels and explicit skipper attributes, including electronics, thereby honing consistent intervessel differences to finer precision. Data more disaggregated than annual data might also capture details that would be otherwise lost.

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Food consumption and growth in maturing Atlantic salmon (*Salmo salar*)

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Abstract: Circulating levels of the steroid hormones 11-ketotestosterone (11-KT) and 17β-estradiol (E2), voluntary food intake, and growth performance were measured in individual Atlantic salmon (*Salmo salar*) over the year preceding their maturation as grilse. 11-KT and E2 were measured by radioimmunoassay, and X-radiography was used to measure food consumption rates. Two phases of sexual maturation were identified: the early phase (October 1992 – April 1993) was characterized by slowly rising steroid hormone levels concomitant with relatively high rates of food consumption and growth, and in the late phase (May–October 1993), steroid hormone levels increased more rapidly and growth rates decreased in association with inappetence. Significant linear relationships were observed between food consumption and specific growth rates of fish throughout the study (ANCOVA, $p < 0.05$). Slopes and intercepts of regressions were similar for fish during early maturation, while a lower intercept and steeper slope (ANCOVA, $p < 0.05$) was observed during the later stages of maturation. In November, January, February, and April, fish in which steroid hormone levels were elevated were significantly heavier than those in which hormone levels were basal. There were no significant differences in specific growth rates (except in February), food consumption, or weight-specific food conversion ratio (except in August and October) associated with maturation status.

Résumé : Le taux circulant des hormones stéroïdiennes 11-cétotestostérone (11-KT) et 17β-oestradiol (E2), l'apport alimentaire volontaire et la performance de croissance ont été mesurés chez des spécimens de saumon atlantique (*Salmo salar*) dans l'année qui a précédé leur passage à l'état de madeleineau. La 11-KT et la E2 ont été dosées par radio-immunoessai, et des radiographies ont permis de mesurer le taux de consommation des aliments. Nous avons identifié deux phases de maturation sexuelle : la phase précoce (octobre 1992 à avril 1993) était caractérisée par une augmentation lente de la concentration des hormones stéroïdiennes et par des taux de consommation des aliments et de croissance assez élevés, et dans la phase tardive (mai à octobre 1993), le taux d'hormone stéroïdienne a augmenté plus rapidement et nous avons relevé une diminution du taux de croissance associée à l'inappétence. Des relations linéaires significatives ont été observées entre la consommation des aliments et le taux de croissance spécifique des poissons tout au long de l'étude (ANCOVA, $p < 0,05$). Les pentes et les points d'intersection des courbes de régression étaient similaires chez les poissons pendant la période de maturation précoce, tandis que des points d'intersection plus bas et une pente plus forte (ANCOVA, $p < 0,05$) ont été notés pendant les dernières étapes de la maturation. En novembre, en janvier, en février et en avril, les poissons chez lesquels le taux d'hormone stéroïdienne était élevé étaient beaucoup plus robustes que ceux qui présentaient un taux d'hormone de base. Le taux de croissance spécifique (sauf en février), la consommation des aliments ou le taux de conversion alimentaire en fonction du poids (sauf en août et en octobre) ne présentaient aucune différence importante associée au stade de maturation.

[Traduit par la Rédaction]

Introduction

Sexual maturation of farmed salmon before harvest in seawater is undesirable to farmers, since it is associated with a cessation in growth and decline in flesh quality, which reduces the market value. In fish, as in other vertebrates, sexual development is endocrine mediated via the hypothalamic–pituitary–gonadal axis. Development of the gonads is under control of hormones from the pituitary, which is in turn controlled by the hypothalamus and by hormone feedback, especially by gonadal steroids (Fostier et al. 1983; Idler and Ng 1983; Peter 1983; Scott 1987; Peter et al. 1991). The principal gonadal steroids of salmon, 17β-estradiol (E2) and 11-ketotestosterone (11-KT), play a physiological role in gonadal differentiation, growth, gametogenesis, and maintenance of somatic tissue (Fostier et al. 1983).

Increases in circulating levels of steroid hormones in salmonids are associated with gametogenesis and gonad development. Specific radioimmunoassays have been used to measure levels of 11-KT and E2 in the blood serum of male and female Atlantic salmon (*Salmo salar*) throughout maturation by Abdullah (1981) and Hunt et al. (1982). These au-

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thors showed that elevated levels of 11-KT and E2 were detectable in some fish up to a year in advance of spawning and prior to the development of overt secondary sexual characteristics. Similarly, in rainbow trout (*Oncorhynchus mykiss*), Schulz (1984) recorded low levels of 11-KT in males prior to the appearance of spermatozoa and maximum values in spermiating males. In female trout, low levels of E2 and testosterone have been measured during the period of slow oocyte growth and maximum values for E2 were determined during vitellogenesis (Scott et al. 1980; Schulz 1984). Therefore, knowledge of levels of steroid hormones over time enables the underlying changes in the reproductive development of fish to be determined without sacrificing the fish.

Reproductive development in salmonids is also associated with changes in somatic growth rate and food intake. Several studies have shown that growth rates of maturing fish peak in the spring and summer in the year of maturation and, over this period, exceed those of their nonmaturing counterparts (Hunt et al. 1982; Youngson et al. 1988; McLay et al. 1992; Tveiten et al. 1996). In contrast, the later stages of maturation are associated with inappetence and loss of body condition (Kadri et al. 1995; Tveiten et al. 1996; Frantzen et al. 1997).

The aim of the present study was to determine how food consumption varies with changes in growth associated with maturation in Atlantic salmon, particularly during the early stages of maturation, which have not been subject to scrutiny. To this end, food consumption, growth performance, and levels of the steroid hormones 11-KT and E2 were measured in individual fish maturing as grilse (after one winter in the sea). Data from the study were also examined to determine whether it was possible to detect differences in food consumption and growth in individual fish prior to endocrine evidence of impending maturity.

Materials and methods

Parentage, maintenance, and feeding of fish

Atlantic salmon used in the study were the progeny of wild salmon, captured prior to spawning at fish traps on the Baddoch and Girnock Burns tributaries of the River Dee in Aberdeenshire, Scotland. Fertilizations using 10 males and 10 females to produce 100 families from each source population were performed in autumn 1989. Fish were reared separately in the Fisheries Research Services hatchery Tullynessle, near Aberdeen, until the end of their first summer. Thereafter, fish were marked with a single Alcian blue dye spot using a Panjet needleless injector (Hart and Pitcher 1969) to denote their origin and reared together for the next 18 months in 1-m-diameter tanks provided with river water. The fish were fed at 3% body weight·day^{-1}. Food was delivered continuously throughout daylight hours using a mechanical feeder.

In April 1992, 50 fish, of which 19 were of Baddoch parentage and 31 of Girnock parentage, were anaesthetized, measured (mean fork length 159 ± 1 mm), and individually marked using unique combinations of Alcian blue dye marks on their ventral body surface. In May 1992, fish were transported to the Fisheries Research Services experimental facility at Aultbea, Wester Ross, and transferred to a 2-m-diameter tank supplied with seawater at a rate of 23 L·min^{-1}. Following transfer, the fish were fed a commercial pelleted diet (BP Nutrition UK Ltd.) at 3% body weight·day^{-1} delivered during daylight hours. From 13 October 1992, to facilitate the measurement of food consumption by X radiography, food delivery was adjusted so that the same ration was fed over a 3-h period between 10:00 and 13:00. Throughout the study, fish were kept under a natural photoperiod and at ambient water temperature.

Assessment of growth and reproductive development

On 13 October 1992, and at approximately monthly intervals thereafter, all fish were anaesthetized using MS 222 (0.2 g·L^{-1}), weighed, measured (fork length), and a 1-mL sample of blood obtained from the caudal vein. On each sampling occasion, blood samples were taken at approximately the same time of day and were refrigerated overnight. Serum was separated by centrifugation and stored at –20°C prior to analysis. Levels of the steroid hormones 11-KT and E2 were determined in 20-µL aliquots of heat-treated serum, diluted 1:10 with phosphate buffer (pH 7.0) serum and heated to 100°C in a water bath for 30 min, using radioimmunoassay procedures described and validated by Youngson et al. (1988). The radiolabelled steroid [2,4,6,7-^{3}H]E2 was purchased from Amersham International and labelled 11-KT was synthesized from [^{3}H]cortisol by R.S. Wright at the Marine Laboratory, Aberdeen, Scotland, as described in Simpson and Wright (1977). Antisera for the E2 assay were purchased from Steranti Research Ltd. 11-KT was assayed using antiserum generated in rabbits (for details, see Simpson and Wright 1977). Values that exceeded the minimum detection limits for the assays, 0.50 ng·mL^{-1} for 11-KT and 0.25 ng·mL^{-1} for E2, were considered to be evidence of impending maturity in males and females, respectively. The sex and extent of reproductive development of fish that died during the course of the experiment were determined by inspection of their gonads. Mortalities were as follows: one immature fish on 3 February, one maturing male on 24 February, maturing females on 18 March (one), 22 June (one), 11 August (one), and 11 females throughout September. Similarly, the sex and maturity status of surviving fish were confirmed at the end of the study on 15 December 1993.

Measurement of food consumption

Starting on 19 January 1993, food consumption was monitored at regular intervals throughout the experiment. When investigating food consumption of individual fish in relation to growth, food consumption was estimated as the mean of three measurements made at approximately biweekly intervals over a defined period of growth (for details, see Stead et al. 1996). In this experiment, three measurements of food consumption were made at approximately 2-week intervals over a 4-week growth period when possible. On the day that consumption was determined, the fish were fed their usual diet, repelleted after the addition of ballotini glass spheres (400–520 µm, 2% wet weight, Jencons Scientific Ltd.). The repelleted diet was prepared as described in Stead et al. (1996) and fed to the fish at the same ration level and for the same duration as the normal diet. One hour after feeding had ceased, groups of between two and five fish were anaesthetized and X rayed using a portable X-ray machine (Todd Research model 20/80) and Kodak Industrex CX film. Based on the measurements of gastric evacuation rates determined by Stead et al. (1996) and the temperatures experienced by the fish in this study, it was assumed that none of the food passed through the alimentary tract of the fish prior to X-ray measurement. Inspection of the X-ray negatives confirmed this, since ballotini glass spheres were observed only in the stomach, and not in the intestine, for up to 6 h after the fish had ceased feeding.

On each sampling occasion, wet weights (grams) and fork lengths (millimetres) were recorded. The number of ballotini glass spheres in the alimentary tract of each fish was counted from the X-ray negatives. The expected weight of food per ballotini glass sphere was calculated using a point estimate: 1 ballotini glass sphere = Σ weight/Σ ballotini glass spheres, where Σ weight represents the sum of labelled subsamples of repelleted food (milli-

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grams of dry food) and Σ ballotini glass spheres is the total number of ballotini glass spheres in previously weighed subsamples. The weight of fish was corrected for the weight of food in the gut by subtracting the dry weight of food consumed by individual fish from the wet weight of fish expressed as milligrams dry food per gram wet weight of fish per day, as described in Stead et al. (1996).

Treatment of data and statistical analyses

Specific growth rates (SGR, percent body weight per day) of individual fish between sampling dates were calculated using the following equation (Ricker 1979):

$$\mathrm{SGR} = \frac{(\ln(W_t) - \ln(W_0)) \times 100}{t}$$

where W_0 and W_t are the initial and final wet weights (grams) of each fish and t is the number of days between weighings.

Food consumption rates determined at approximately biweekly intervals throughout the experiment were used to calculate weight-specific food conversion ratios (FCR, milligrams per gram) (Stead et al. 1996) using

$$\mathrm{FCR} = \frac{(\text{food consumption} \times t)}{W_t - W_0}$$

where food consumed (milligrams dry food per gram wet weight of fish per day) is the food consumption data derived from each sampling occasion (approximately biweekly) by X radiography (for validation of method, see Stead et al. 1996), W_t is the wet weight (grams) at time t, W_0 is the initial wet weight (grams), and t is the number of days between weighings.

Condition factors (K, grams per cubic centimetre) were determined according to the formula

$$K = \frac{W}{L^3} \times 100$$

where W is wet weight (grams) and L is final fork length (centimetres).

To determine the time when endocrine evidence of maturity was first detected for each fish, the best line was fitted through the individual male and female hormone level data, subject to it never decreasing, using the monotonic regression facilities of the Genstat 5 statistical package (Payne et al. 1993). The Wilcoxon test was then applied to this data to test for any differences in timing of onset of maturation in Girnock and Baddoch males and between Girnock and Baddoch females.

The antedependence method of Kenward (1987) was employed to investigate differences in steroid hormone levels (11-KT and E2), lengths, weights, specific growth rates, food consumption rates, weight-specific food conversion ratios, and condition factors in males and females and within and between stocks. Essentially, this tests for differences in a variable, for example, growth rate, at a particular point in time, independent of any differences that have been identified previously. Because of small sample sizes, data from fish that remained immature were excluded from these analyses.

The relationship between individual specific growth rates and food consumption at different stages of maturation was examined by linear regression. Slopes and intercepts during two periods early in maturation (19 January – 10 March 1993, 29 April – 13 May 1993) and late in maturation (August–October 1993) were compared using analysis of covariance (ANCOVA) with food consumption as the covariate. Food consumption estimates, based on the means of two or three X-ray measurements depending on the length of the growth period examined, were compared with specific growth rates over the same time period.

At each sampling date, the weight, specific growth rates, food consumption, and weight-specific food conversion ratios of fish showing endocrine evidence of maturation, fish in which hormone levels were basal, and others that remained immature were compared using the Kruskal–Wallis test.

Results

Steroid hormone levels

When blood was first sampled on 13 October 1992, one male fish had levels of 11-KT in excess of 0.5 ng·mL^{-1}. Hormone levels were elevated in a further five male fish in November and in another three males in December, and all of the males had shown endocrine evidence of having started to mature by the February sampling date (Table 1). The first elevations of E2 in females were detected over a more protracted period. Three females had levels of E2 in excess of 0.25 ng·mL^{-1} in October. E2 levels were elevated in 27 fish by February and in all females (n = 34) by May (Table 1). Although all fish showing elevated hormones levels in October were of Girnock parentage, differences in the timing of changes in Girnock and Baddoch males and Girnock and Baddoch females were not significant.

Between October 1992 and April 1993, mean steroid hormone levels increased slowly in maturing fish (Fig. 1). Levels of 11-KT in males showed a small peak between January and March, which is apparent in the summarized data (Fig. 1). In the majority of fish, steroid hormone levels increased more rapidly after May 1993 to reach maximum levels in October 1993 (Fig. 1). Levels of 11-KT and E2 remained at or below the detection limit of the assay (0.50 ng·mL^{-1} for 11-KT and 0.25 ng·mL^{-1} for E2) in the four fish that remained immature. At the end of the experiment, visual inspection confirmed the sex and maturation status of fish, inferred from steroid hormone measurements.

Growth and food consumption

Specific growth rates, food consumption, weight-specific food conversion ratios, and condition factors in maturing males and females are summarized in Figs. 2–5. Antedependence analysis detected no significant differences in these parameters or in the lengths and weights (data not shown) between males and females or between Baddoch and Girnock stocks.

Between October 1992 and April 1993, mean specific growth rates were high compared with values observed between May and October 1993 (Fig. 2). From May, growth rates decreased and were negative from July onwards as fish lost weight (Fig. 2). Between January and April, mean food consumption rates were variable but higher than those recorded from May onwards (Fig. 3). Weight-specific food conversion ratios showed a pattern generally similar to that of consumption (Fig. 4). Condition factors were variable in the overwinter period, rose to a peak in April, and declined thereafter (Fig. 5).

Significant linear relationships were observed between food consumption and specific growth rates determined for individual fish (ANCOVA, $p < 0.05$) (Fig. 6). The slopes and intercepts of the regression lines for data from 19 January to 10 March 1993 (Fig. 6*a*) and between 29 April and 13 May (Fig. 6*b*) were not significantly different. The intercept of

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Table 1. Monthly variation in wet weight (g), specific growth rate (% body weight·day^{-1}), food consumption (mg dry food·g wet weight fish^{-1}·day^{-1}), and weight-specific food conversion ratio (mg·g^{-1}).

Month		*n*	Weight	Specific growth rate	Food consumption	Food conversion ratio
October	imm	4	267 (192–309)			
	>♂	1	349			
	<♂	11	274 (173–322)			
	>♀	3	249 (240–283)			
	<♀	31	247 (130–322)			
November	imm	4	299 (258–343)*	0.5 (0.0–0.8)		
	>♂	6	379 (351–502)*	0.8 (0.7–1.0)		
	<♂	6	246 (200–393)*	0.6 (0.4–1.0)		
	>♀	6	302 (287–393)*	0.6 (0.4–0.7)		
	<♀	28	303 (153–383)*	0.5 (0.3–0.9)		
December	imm	4	319 (272–417)	0.2 (–0.1–1.3)		
	>♂	9	400 (209–519)	0.5 (0.1–0.7)		
	<♂	3	340 (249–423)	0.2 (0.1–0.3)		
	>♀	15	352 (187–433)	0.4 (0.1–0.7)		
	<♀	19	304 (163–400)	0.4 (–0.3–1.1)		
January	imm	4	348 (273–480)*	0.3 (–0.1–0.3)	0.8 (0.2–1.2)	0.7 (–1.0–1.8)
	>♂	11	499 (234–635)*	0.6 (0.3–0.7)	1.6 (0.3–4.2)	0.9 (0.2–1.2)
	<♂	1	406*	0.4	0.8	0.5
	>♀	25	416 (186–537)*	0.5 (0.3–0.7)	1.5 (0.4–3.6)	0.8 (0.4–3.7)
	<♀	9	321 (248–409)*	0.5 (–0.1–0.7)	1.0 (0.0–1.9)	0.9 (0.0–1.3)
February	imm	3	401 (317–494)*	0.1 (0.1–0.2)*	0.7 (0.7–1.5)	2.4 (1.0–4.3)
	>♂	12	519 (247–690)*	0.4 (0.2–0.7)*	1.8 (0.7–5.5)	1.2 (0.5–1.9)
	<♂					
	>♀	27	447 (199–587)*	0.4 (–0.2–0.9)*	1.3 (0.3–4.6)	0.9 (–1.5–3.3)
	<♀	7	377 (240–436)*	0.3 (–0.1–0.5)*	1.1 (0.0–2.1)	0.9 (0.0–1.8)
March	imm	3	412 (320–531)	0.1 (0.0–0.3)	0.1 (0.0–0.3)	0.1 (0.0–2.3)
	>♂	11	588 (311–774)	0.4 (0.2–0.9)	1.1 (0.4–2.7)	0.5 (0.1–1.6)
	<♂					
	>♀	29	508 (224–675)	0.5 (0.1–0.8)	1.0 (0.2–3.3)	0.5 (0.1–1.4)
	<♀	5	405 (251–574)	0.5 (0.2–1.1)	1.5 (0.1–2.4)	0.4 (0.1–0.7)
April	imm	2	394 (347–441)*	0.2 (0.2–0.2)	0.9 (0.2–1.5)	0.7 (0.0–1.3)
	>♂	11	683 (454–884)*	0.4 (0.2–1.1)	1.8 (0.0–5.1)	0.6 (0.0–3.3)
	<♂					
	>♀	29	603 (273–887)*	0.6 (0.2–0.9)	1.8 (0.0–6.4)	0.6 (0.0–1.4)
	<♀	29	495 (357–681)*	0.5 (0.3–1.0)	1.6 (0.6–5.7)	0.8 (0.2–3.3)
May	imm	2	402 (350–453)	0.1 (0.0–0.1)	0.4 (0.0–0.9)	0.6 (0.0–1.2)
	>♂	11	682 (466–920)	0.2 (–0.1–0.4)	0.1 (0.0–1.9)	0 (–2.2–0.0)
	<♂					
	>♀	34	591 (291–948)	0.2 (–0.3–1.0)	0.1 (0.0–1.6)	0 (–1.5–3.5)
	<♀					
August	imm	2	541 (522–559)	0.5 (0.5–0.6)*	1.1 (0.8–1.4)	0.4 (0.3–0.5)*
	>♂	11	683 (411–1047)	–0.2 (–0.3–0.3)*	0.0 (0.0–2.1)	0.0 (0.0–0.7)*
	<♂					
	>♀	32	623 (296–948)	–0.2 (–0.4–0.3)*	0.0 (0.0–0.9)	0.0 (–0.2–0.7)*
	<♀					
October	imm	2	808 (730–885)	0.6 (0.5–0.7)	0.5 (0.0–1.0)	0.1 (0.0–0.2)*
	>♂	11	620 (365–1133)	–0.2 (–0.2–0.1)	0.0 (0.0–0.0)	0.0 (0.0–0.0)*
	<♂					
	>♀	21	622 (250–897)	–0.2 (–0.3–0.3)	0.0 (0.0–1.4)	0.0 (0.0–0.7)*
	<♀					

Note: Data are medians and range (minimum–maximum) for each sampling date for fish that remained immature (imm), males that matured as grilse in which 11-KT levels were >0.5 ng·mL^{-1} (>♂), males that matured as grilse in which 11-KT levels were <0.5 ng·mL^{-1} (<♂), females that matured as grilse in which E2 levels were >0.25 ng·mL^{-1} (>♀), and females that matured as grilse in which E2 levels were <0.25 ng·mL^{-1} (<♀); *n* is the number of fish in each category at each sampling date and an asterisk denotes significant differences between groups at particular sampling dates (Kruskal–Wallis, $p < 0.05$).

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Fig. 1. Serum 11-KT levels in maturing male (■, n = 12) and E2 levels in maturing female (○, n = 34) Atlantic salmon between October 1992 and October 1993. Data are means ± SE.

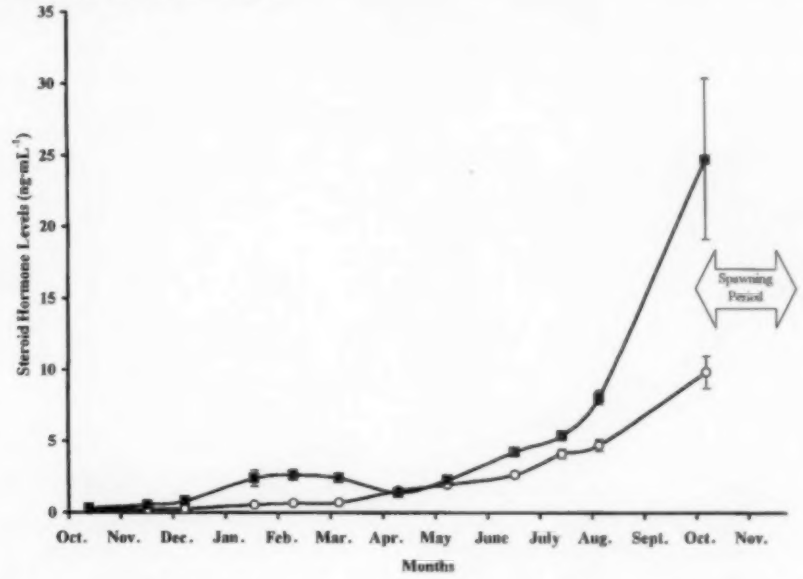
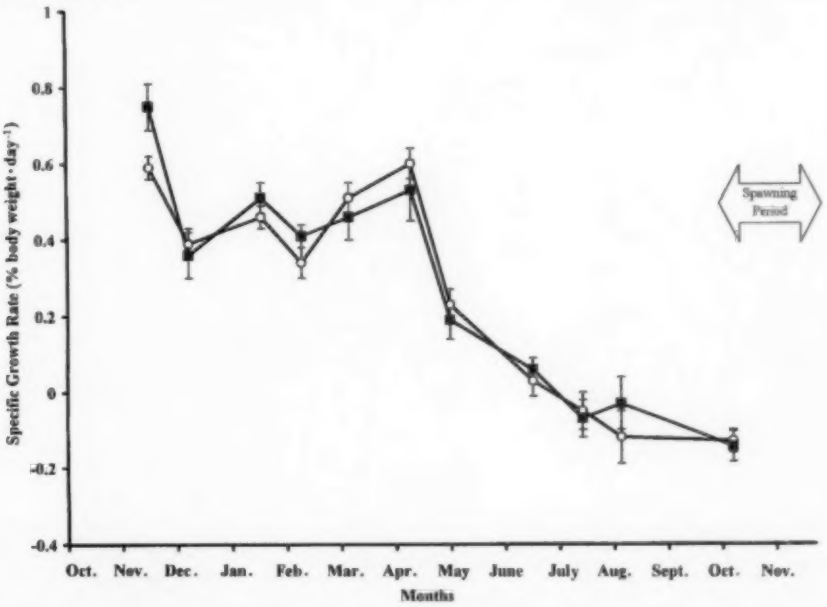


Fig. 2. Specific growth rates of maturing male (■, n = 12) and female (○, n = 34) Atlantic salmon between October 1992 and October 1993. Data are means ± SE.



the regression line for fish measured between 11 August and 29 October 1993 (Fig. 6*c*) was lower and the slope was steeper than for those fish measured earlier in the study (ANCOVA, $p < 0.05$) (Figs. 6*a* and 6*b*). Between 19 January and 10 March 1993, data points for immature fish lay below the fitted relationship (Fig. 6*a*). In April and May 1993, the two remaining immature fish had rates of growth similar, for a given consumption rate, to maturing fish (Fig. 6*b*). Finally, from 11 August until 29 October 1993, the immature fish generally had higher consumption rates with correspondingly higher growth rates than sexually mature fish (Fig. 6*c*).

Comparisons of size, food consumption, and growth of maturing and nonmaturing fish

Differences in weight, growth, food consumption, and weight-specific food conversion ratios were considered in re-

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Fig. 3. Food consumption rates of maturing male (■, n = 12) and female (○, n = 34) Atlantic salmon between October 1992 and October 1993. Data are means ± SE.

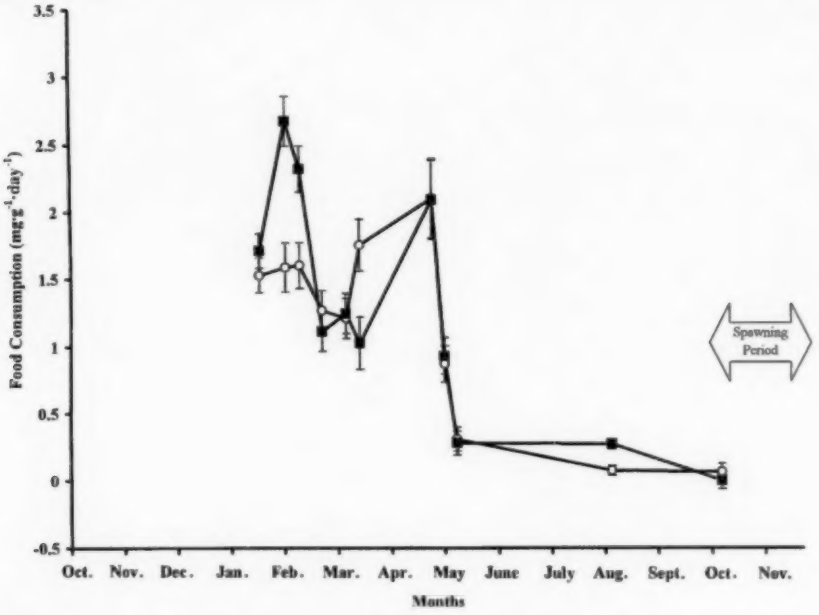
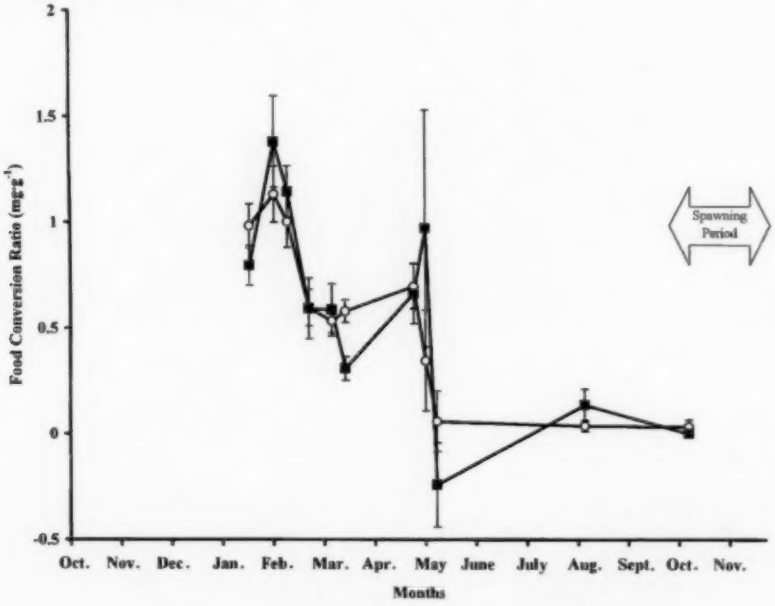


Fig. 4. Weight-specific food conversion ratios of maturing male (■, n = 12) and female (○, n = 34) Atlantic salmon between October 1992 and October 1993. Data are means ± SE.



lation to the time at which individual fish first showed hormonal evidence of having started to mature (Table 1). At the start of the study in October 1992, the single male fish in which 11-KT levels exceeded 0.5 ng·mL^{-1} was the heaviest of the group, but the weights of females in which E2 levels were elevated (>0.25 ng·mL^{-1}) were not significantly different from those in which levels were basal (<0.25 ng·mL^{-1}) or the median weight of fish that remained immature. In November the median weight of male fish in which hormone levels were elevated was greater ($p < 0.05$) than that of other males and that of females and fish that remained immature. Similarly, between January and April, median weights of

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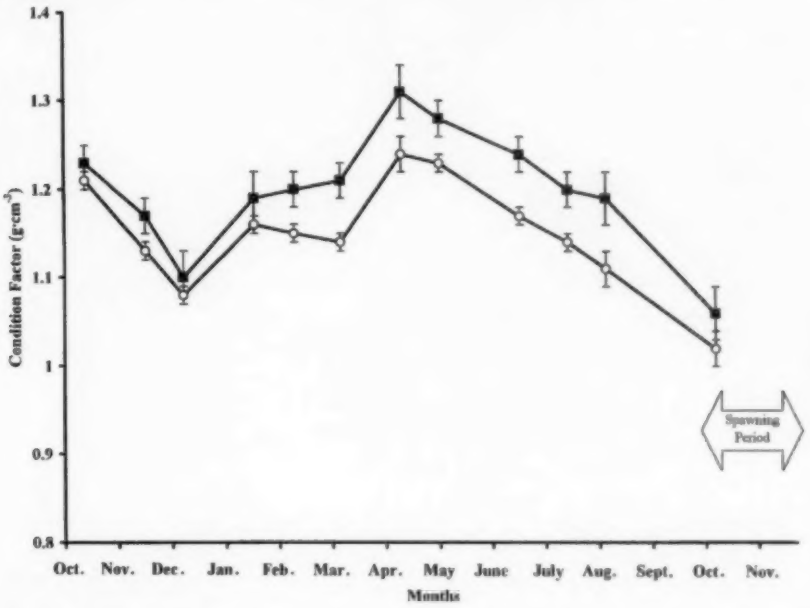
Fig. 5. Condition factors of maturing male (■, n = 12) and female (○, n = 34) Atlantic salmon between October 1992 and October 1993. Data are means ± SE.



fish of both sexes in which hormone levels were elevated exceeded those of fish in which levels were basal; differences were significant for all sampling dates except March (p < 0.05). Between November 1992 and April 1993, median specific growth rates were generally higher in fish in which hormone levels were elevated, but differences between groups were only significant in February (p < 0.05) when the lowest specific growth rates were evident in immature fish. Food consumption and weight-specific food conversion ratio, measured between January and May, did not vary significantly in relation to maturation status (Table 1). Between June and July, there were no differences in weight and growth rate of maturing and nonmaturing fish (data not shown). From August onwards, growth rates, food consumption, and weight-specific food conversion ratio in fish that remained immature were higher than in maturing fish that by this time had ceased feeding and were losing weight (see Fig. 6*c*).

Discussion

Based on the summarized measurements of growth, food consumption, and steroid hormone levels made in maturing fish in this study, it was possible to distinguish two phases of maturation. The first or early phase, spanning the period between October 1992 and April 1993, was characterized by initial increases in circulating steroid hormone levels. From January onwards, both males and females had similar but relatively high rates of food consumption and growth. During this period, steroid hormone levels were rising but did not exceed 3 ng·mL^{-1} in either males or females. From May onwards, fish entered a second or late phase of maturation during which circulating steroid hormone levels increased more rapidly to reach maximum recorded values in October 1993. Over this period, growth rates declined in association with inappetence. These changes, however, did not occur simultaneously in all individuals.

Circulating levels of 11-KT in excess of 0.5 ng·mL^{-1} and levels of E2 greater than 0.25 ng·mL^{-1} were measured in some individuals when first sampled in October 1992. All males had shown endocrine evidence of impending maturity by February 1993. Elevations of E2 were detected somewhat later in females, but the majority had shown evidence of having started to mature by May. For some males, levels of 11-KT reached a minor peak in spring. However, this was not the case with the females, in which E2 levels rose steadily following their first detection to reach their maximum values in October 1993. These findings are similar to those of McLay et al. (1992) who reported a minor peak in 11-KT levels in male grilse in February of the same year in which the fish later matured but no evidence of an early spring peak in E2 in female grilse. Early spring peaks in E2 have been found to occur in female salmon (Abdullah 1981; Youngson et al. 1988), and Hunt et al. (1982) also reported an early peak in 11-KT in male grilse in March. Based on the observation that elevations of hormone levels were invariably associated with sexual maturation in the same year, Abdullah (1981) postulated that the physiological decision to mature must be taken during the late winter or spring preceding spawning. Data from this study support this but suggest that in some individuals, the onset of reproductive development may occur even earlier.

During the early phase of maturation, food consumption and growth rates, although variable, were relatively high. Abdullah (1981), Hunt et al. (1982), Youngson et al. (1988), and McLay et al. (1992) have recorded peak growth rates during this early phase of maturation for male and female Atlantic salmon. Abdullah (1981) noted that the increase in growth rates could not be explained by temperature, even

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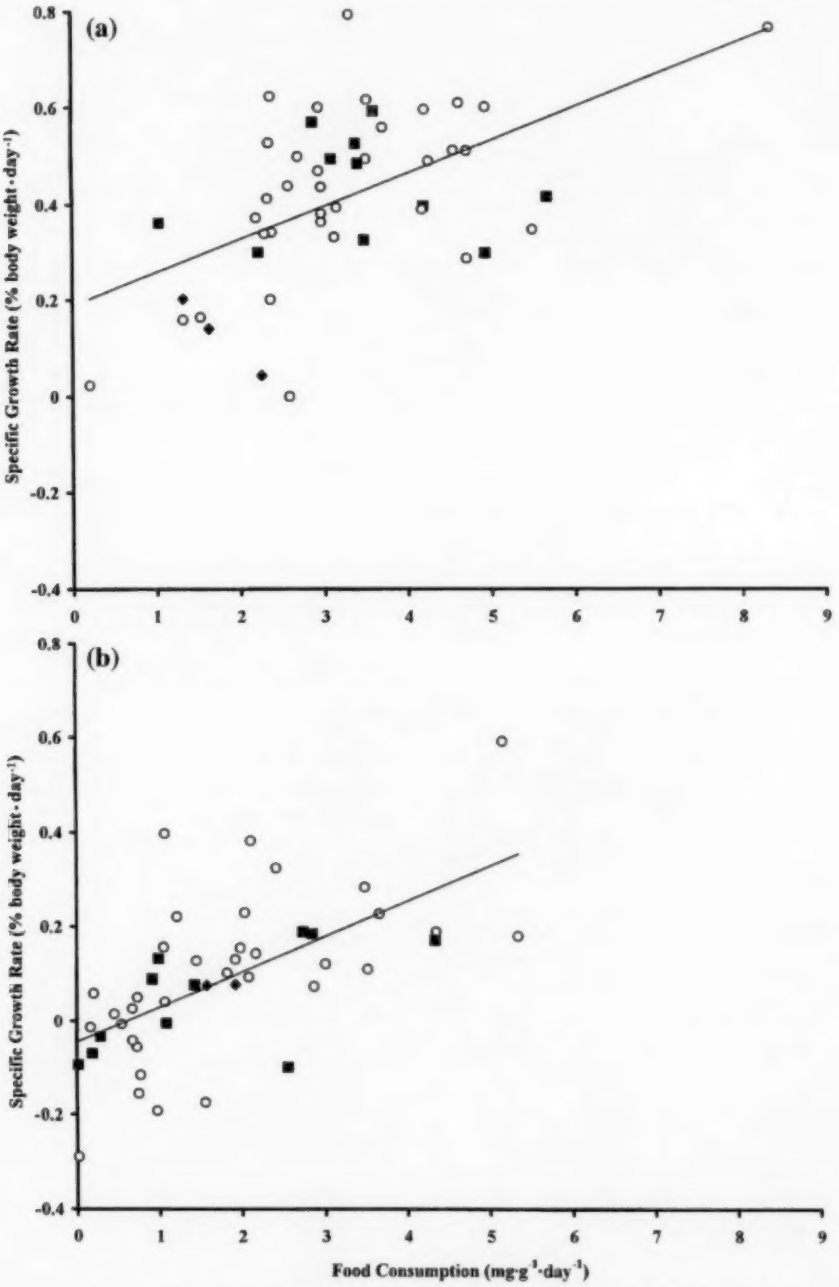
Fig. 6. Relationship (linear regression) between food consumption and specific growth rates in the period (*a*) 19 January – 10 March 1993, (*b*) 29 April – 13 May 1993, and (*c*) 11 August – 29 October 1993 for maturing male (■), maturing female (○), and immature (◆) Atlantic salmon.



though this increase corresponded to increasing water temperature, because sharp changes in growth were observed when the temperature of the water remained steady. Similarly, in this study, changes in growth rate did not reflect ambient water temperatures; the highest growth rates were observed between October and April whereas water temperatures peaked in July and August.

It has been suggested that the high rates of food consumption observed early in maturation are required to achieve sufficient body reserves to support gametogenesis (Tveiten et al. 1996). In this study, weight-specific food conversion ratios were high in January and February, values close to 1 indicating efficient use of feed for weight gain. Weight-specific food conversion ratios declined from March on-

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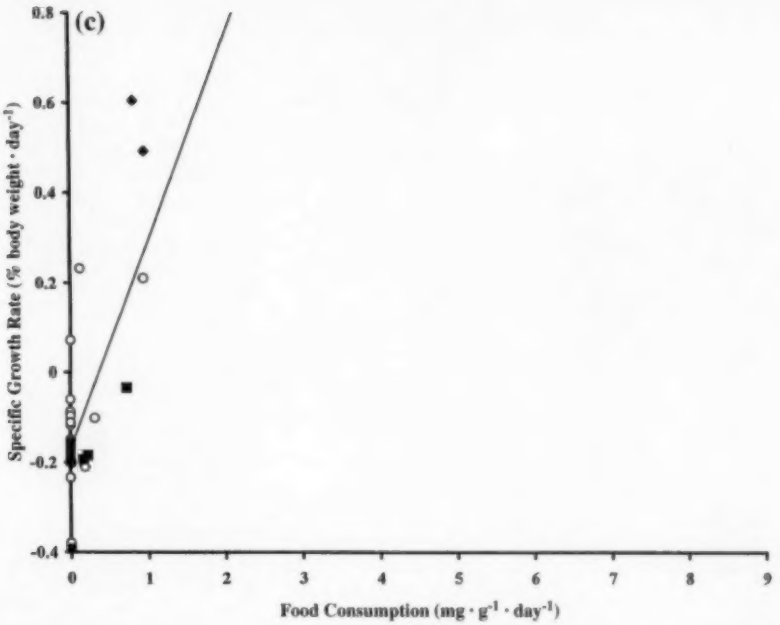
Fig. 6 (*concluded*).



wards, the lower values indicating that the efficiency with which feed was being utilized for growth was lower. Low weight-specific food conversion ratios could result from a reduction in feeding, as is known to occur in maturing salmonids (Kadri et al. 1995; Tveiten et al. 1996, 1998; Damsgård et al. 1999), and (or) from a preferential deposition of lipid rather than lean tissue, e.g., locomotor muscle. It is tempting to speculate that the high feeding rates accompanied by high weight-specific food conversion ratios seen early in the year reflected the nutrient accumulation that would later be remobilized for gonadal development. However, the exact relationships among food consumption, weight gain, energy deposition, and food conversion ratio in maturing fish remain to be investigated.

In the present study, food consumption rates and growth declined from May onwards as fish entered the second stage of maturation. Similarly, Abdullah (1981) found that in the summer preceding spawning, both grilse and salmon that matured after two winters in seawater suffered sharp decreases in growth rates and eventually lost weight. Kadri et al. (1995) observed a depression in appetite among maturing Atlantic salmon prior to spawning, and this is substantiated by the consumption measurements made in this study. Although some fish continued to feed beyond May, these were for the most part those that remained immature throughout the study. Other studies have shown that growth of maturing fish slows from July of the year in which sexual maturity is attained at a time at which energy is diverted into gonad production (Lundqvist and Fridberg 1982; Skilbrei 1989; Rowe and Thorpe 1990). Kadri et al. (1995) suggested that the onset of anorexia in maturing female Atlantic salmon is related to the attainment of critical threshold values of lean body mass and, to a lesser extent, body lipid levels and gonadosomatic index. Maturing Arctic char (*Salvelinus alpinus*) appeared to reduce feeding once threshold condition factors of 1.4–1.5 had been reached (Tveiten et al. 1996). Similarly, in this study, condition factors reached a peak at 1.3 prior to the observed decline in food consumption rates.

To examine the role of growth in the onset of maturation, the size and growth rates of fish showing hormonal evidence of maturation onset were compared with those in fish in which hormone levels were basal and with those in the small number of fish that remained immature. It was found that fish in the early stages of maturation were, for the most part, larger than fish in which hormone levels were basal. Differences in growth rate were only occasionally significant. Given that the first time that steroid hormone levels were measured the bigger fish had higher steroid levels, it is not possible from the results of this study to determine whether steroid levels brought on faster growth or vice versa. Gjerde (1984) questioned whether the observed differences in size between maturing and nonmaturing Atlantic salmon were due to growth affecting the onset of maturation or whether maturation accelerated growth. Overall, the findings of the present study provide more evidence that growth and sexual maturity are closely linked processes and that this makes it difficult to determine if growth induces maturity or vice versa. Several authors have suggested that low levels of steroid hormones may accelerate growth early in maturation (LeGac et al. 1993), but it is not clear whether any anabolic effect is due to increased food consumption or changes in conversion efficiency. The relationship between growth rate and food consumption observed during winter and early spring suggests that the acceleration of growth in early maturation is at least in part related to the amounts of food consumed. Over this period the highest weight-specific food conversion ratios were observed in fish in which hormone levels were elevated, but differences were not significant. Further and more targeted work would be necessary to establish whether the hormonal changes associated with matu-

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ration affect food conversion. The present study does, however, contribute to knowledge of changes in food consumption and growth in salmon in the year leading up to sexual maturity.

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Concordance of taxonomic composition patterns across multiple lake assemblages: effects of scale, body size, and land use

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Abstract: We assessed environmental gradients and the extent to which they induced concordant patterns of taxonomic composition among benthic macroinvertebrate, riparian bird, sedimentary diatom, fish, and pelagic zooplankton assemblages in 186 northeastern U.S.A. lakes. Human population density showed a close correspondence to this region's dominant environmental gradient. This reflected the constraints imposed by climate and geomorphology on land use and, in turn, the effects of land use on the environment (e.g., increasing lake productivity). For the region as a whole, concordance was highest among assemblages whose taxa were relatively similar in body size. The larger-bodied assemblages (benthos, birds, fish) were correlated most strongly with factors of broader scale (climate, forest composition) than the diatoms and zooplankton (pH, lake depth). Assemblage concordance showed little or no relationship to body size when upland and lowland subregions were examined separately. This was presumably because differences in the scales at which each assemblage integrated the environment were obscured more locally. The larger-bodied assemblages showed stronger associations with land use than the diatoms and zooplankton. This occurred, in part, because they responded more strongly to broad-scale, nonanthropogenic factors that also affected land use. We argue, however, that the larger-bodied assemblages have also been more severely affected by human activities.

Résumé : Nous avons évalué les gradients environnementaux et la mesure dans laquelle ils induisaient des concordances de la composition taxonomique au sein de communautés de macroinvertébrés benthiques, d'oiseaux de rivage, de diatomées des sédiments, de poissons et d'organismes zooplanctoniques pélagiques de 186 lacs du nord-est des États-Unis. La densité de la population humaine présentait une correspondance étroite avec le principal gradient environnemental de la région. Cela reflétait les contraintes imposées par le climat et la géomorphologie sur l'utilisation des terres et, ensuite, les effets de l'utilisation des terres sur l'environnement (p. ex. : accroissement de la productivité des lacs). Dans l'ensemble de la région, la concordance était plus élevée entre les communautés dont les taxons étaient de taille relativement semblable. Dans le cas des communautés à individus plus gros (benthos, oiseaux, poissons), la corrélation était plus forte avec des facteurs agissant sur une plus grande échelle (climat, composition de la forêt) que chez les diatomées et le zooplancton (pH, profondeur des lacs). La concordance des communautés présentait peu ou pas de rapports avec la taille lorsque les sous-régions de hautes terres ou de basses terres étaient examinées séparément. Cela s'explique sans doute par le fait que les écarts entre les échelles où chaque communauté s'intègre à l'environnement étaient plus obscurcis localement. Les communautés d'individus plus gros présentaient de plus fortes corrélations avec l'utilisation des terres que celles des diatomées et du zooplancton notamment parce qu'elles réagissaient plus fortement à des facteurs non anthropogènes et de plus grande échelle qui affectaient aussi l'utilisation des terres. Nous soutenons cependant que les communautés d'individus plus gros ont aussi été plus sévèrement affectés par les activités humaines.

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Introduction

Studies in community ecology often focus on one taxonomically restricted assemblage of organisms (e.g., fish, diatoms, or birds), interspecific interactions among members of the assemblage, and environmental gradients to which the assemblage responds (Jackson and Harvey 1993). This approach limits the scope of interpretation to restricted taxonomic groups. It also ignores the fact that disparate taxa may interact in ways that affect ecosystem processes (Carpenter and Cottingham 1997). The concordance among different assemblages at the same locations may yield new insights by highlighting similarities and differences in how different kinds of organisms respond to the environment. For example, Jackson and Harvey (1993) observed pronounced differences in the environmental gradients integrated by fish and benthic macroinvertebrates in 40 lakes of south-central Ontario. However, taxonomic composition patterns for the two assemblages were significantly correlated, suggesting biological interactions among the two groups.

The simultaneous consideration of diverse taxa provides a more complete view of human effects on ecosystems because human activities alter the environment in multiple ways at diverse scales (Carpenter and Cottingham 1997). Anthropogenic factors affecting lake biota of the northeastern United States include industrial air pollution (Dixit et al. 1999), land use (Allen and O'Connor 1999), and introductions of nonnative species (Stemberger 1995; Whittier et al. 1995, 1997; Whittier and Kincaid 1999).

Taxa may be differentially affected by human activity to the extent that they respond to different environmental features. Croonquist and Brooks (1991) reported that birds were more sensitive than mammals to human-induced disturbance of riparian wetland areas in a Pennsylvania watershed, perhaps because the birds were more responsive to changes in vegetation structure. Lammert and Allan (1999) observed that stream fish were more sensitive than macroinvertebrates to land use in three tributaries of a river in southeastern Michigan. Allen et al. (1999) found that associations of taxonomic richness with land use varied from positive to negative for disparate assemblages in 186 northeastern U.S.A. lakes, reflecting differences in the anthropogenic factors integrated by each assemblage.

The objective of this study was to identify similarities and differences in the environmental gradients integrated by benthic macroinvertebrates, riparian birds, sedimentary diatoms, fish, and pelagic zooplankton in 186 northeastern U.S.A. lakes. To do so, we used ordination, a technique that identifies the dominant environmental gradients for an assemblage by ordering sites so as to maximize between-site differences in taxonomic composition (ter Braak 1987*a*). We assessed the extent to which different assemblages were influenced by similar factors (i.e., concordant) by separately ordinating data for each assemblage and then comparing how the same sites were ordered in the different ordinations. This work follows an evaluation of taxonomic richness concordance for these same assemblages and lakes (Allen et al. 1999).

Methods

Sampling design and data collection

The data were collected between 1991 and 1995 from 186 northeastern U.S.A. lakes by the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP, Paulsen and Linthurst 1994). The lakes were randomly selected using a probability design to be representative of regional conditions (Larsen et al. 1994). The number of lakes analyzed ranged from 127 for the benthos to 186 for the birds; 114 of these lakes had data for all five assemblages. We analyzed assemblage and habitat data collected during a single visit to each lake. Many of these data are available at the EMAP website (http://www.epa.gov/emap/html/datal/surfwatr/data/nelakes).

A field ornithologist recorded all aquatic and terrestrial birds seen or heard within a 100-m radius during 5-min point counts (Baker et al. 1997). Counts were conducted a minimum of 200 m apart by canoeing a transect 10 m from and parallel to the lakeshore beginning just after sunrise. The bird data were collected between late May and early July, whereas most other data were collected in July and August. Benthic macroinvertebrates were collected by corer from sublittoral sediments at 10 evenly spaced locations along the lake perimeter. We analyzed annelid worms and chironomid midges identified to genus but not other taxa because they were collected infrequently and identified to a coarser and more variable taxonomic resolution. Surficial sediment diatoms were collected by corer at the deepest portion of the lake and identified to the lowest taxonomic resolution (usually species or lower). Fish were collected using gill nets, trap nets, minnow traps, and beach seines to obtain a sample representative of the entire lake. We analyzed native and nonnative fish species, excluding those maintained only by stocking. Zooplankton were collected using a single vertical net tow in the deepest portion of the lake. We analyzed standardized abundance data (individuals per litre) for crustacean and rotifer species. Methods used to process the biological samples are summarized in Allen et al. (1999).

We selected 35 environmental variables for analysis (Table 1) based on results of previous EMAP investigations (e.g., Stemberger and Lazorchak 1994; Whittier et al. 1997; Allen et al. 1999; Dixit et al. 1999). These variables characterized the lake (morphology, water quality, riparian – littoral zone structure), its watershed, and the surrounding landscape. Data on lake water quality and riparian – littoral zone structure were collected by EMAP (Baker et al. 1997). We obtained data from a variety of sources to estimate the proportions of each watershed in various land uses (land use – land cover classification (LULC), Anderson et al. 1976) and to estimate human population density, road density (U.S. Bureau of Census 1990), and point-source pollution density (Abramovitz et al. 1990) for each watershed. Landscape compositional measures derived from AVHRR satellite imagery and the Digital Chart of the World were spatially registered to a 640-km^2 grid of hexagons whose centers were 27 km apart (O'Connor et al. 1996). A seasonal climatic index (seasonality, difference between July and January mean temperatures) derived from the Historical Climate Database was also registered to this hexagon grid. Values for these landscape compositional and climatic measures were assigned as attributes to lakes present in each hexagon. The hexagon data classified lands somewhat differently than LULC and at a coarser resolution. Environmental variables were transformed as necessary to normalize their distributions prior to analysis.

Statistical approach

Characterizing environmental gradients

We performed a principal components analysis (PCA) on the correlation matrix derived from the 35 environmental variables to characterize environmental gradients for the region's lakes and watersheds and to quantify covariation among anthropogenic and nonanthropogenic variables. PCA axis scores were correlated with the 35 variables from which they were derived to aid in interpretation (Table 1).

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Table 1. Correlations (r) of the first two principal component axes with the 35 environmental variables from which they were derived, along with minimums, medians, and maximums of the 35 variables in their original units.

Variable		Untransformed values			r	
Feature	Attribute	Minimum	Median	Maximum	PC1	PC2
Watershed	Human population density (km^{-2})[a]	0	9.5	1760	**0.90**	–0.18
Water quality	Chloride (mequiv.·L^{-1})[b]	5	110	5325	**0.85**	–0.26
Landscape	Seasonality (°C)[c]	23.3	27.2	30.4	**–0.77**	0.06
Landscape	Mixed-conifer forest (%)[c]	0	24	99	**–0.75**	0.07
Watershed	Urban (%)[d]	0	0	94	**0.72**	–0.13
Landscape	Urban (%)[c]	0	0	55	**0.71**	–0.03
Watershed	Point-source pollution (km^{-2})[e]	0	0.02	2.05	**0.70**	–0.13
Watershed	Road density (km·km^{-2})[a]	0	12	102	**0.67**	–0.17
Landscape	Elevation (m)	2	211	700	**–0.60**	0.01
Water quality	Total phosphorus (mg·L^{-1})[b]	0	10	176	**0.58**	**0.59**
Shoreline	Conifer forest (%)[b]	0	17	100	**–0.56**	0.34
Water quality	Sulfate (mequiv.·L^{-1})[b]	27	103	8500	**0.55**	–0.35
Water quality	Turbidity (NTU)[b]	0.2	0.8	14	**0.54**	**0.45**
Landscape	Deciduous forest (%)[c]	0	16	98	**0.52**	–0.22
Water quality	Calcium (mequiv.·L^{-1})[b]	35	205	9004	**0.51**	–0.19
Water quality	Chlorophyll *a* (mg·L^{-1})[b]	0.3	3.8	191.9	**0.49**	**0.55**
Water quality	Total nitrogen (mg·L^{-1})[b]	153	331	1770	**0.48**	**0.63**
Watershed	Agriculture (%)[d]	0	1	90	**0.48**	–0.07
Shoreline	Deciduous forest (%)[b]	0	43	100	**0.47**	–0.09
Shoreline	Residential (%)[b]	0	13	100	**0.44**	–0.31
Shoreline	Mixed forest (%)[b]	0	46	100	**–0.44**	–0.22
Shoreline	Agriculture (%)[b]	0	0	100	**0.43**	0.07
Lake morphology	Maximum depth (m)[b]	1	6.4	47.2	–0.28	**–0.81**
Water quality	Dissolved organic carbon (mg·L^{-1})[b]	1.7	4.4	18.2	–0.09	**0.75**
Water quality	Minimum water temperature (°C)[b]	0	16	29	0.35	**0.66**
Shoreline	Wetland (%)[b]	0	17	100	–0.01	**0.57**
Littoral zone	Substrate size (ordinal)[b]	1	1.7	4.9	–0.25	**–0.56**
Water quality	Total aluminum (mg·L^{-1})[b]	0	15	280	–0.36	**0.46**
Water quality	pH[b]	4.4	7.08	9.05	0.27	**–0.41**
Lake morphology	Area (ha)	0.6	29	3306	–0.37	–0.38
Watershed	Wetland (%)[d]	0	0	29	–0.25	0.30
Water quality	Minimum dissolved oxygen (mg·L^{-1})[b]	0	3.5	8.6	–0.17	0.29
Littoral zone	Macrophytes (no.·$stop^{-1}$)[b]	0	1.3	3	0.07	0.23
Landscape	Agriculture (%)[c]	0	23	88	0.37	0.13
Water quality	Silica (mg·L^{-1})[b]	0	2	15	0.06	0.02
Variance explained (%)					26	14

Note: LUIs are underlined in contrast with the OTHERs. Environmental variables are successively grouped and sorted on the axes where their influence appears most prominent (denoted by boldface type), but the cutoff used to assess influence ($|r| \geq 0.40$) is arbitrary. Data sources or collection methods are footnoted.

[a]U.S. Bureau of Census (1990).
[b]Baker et al. (1997).
[c]O'Connor et al. (1996).
[d]Anderson et al. (1976).
[e]Abramovitz et al. (1990).

Relating assemblage data to environmental data

We constrained variation in the assemblage data to linear combinations of environmental variables using canonical correspondence analysis (CCA) in CANOCO 3.10 (ter Braak 1987*b*), thereby directly associating taxonomic composition with multivariate environmental gradients (ter Braak 1987*a*). CCA assumes that taxa show unimodal responses to the defined environmental gradients, but the technique is robust to departures from this assumption. Rare taxa were down-weighted to reduce their influence in the ordination. Abundances for all assemblages were transformed ($\log_e(y + 1)$) prior to CCA.

In order to assess the relative strengths of assemblage associations with anthropogenic and nonanthropogenic factors, we first divided the 35 environmental variables into two groups for analysis: variables pertaining directly to land use (land use indicators or LUIs) and other environmental measures (OTHERs) (Table 1). We then independently submitted all LUIs and OTHERs to CCA (along with each assemblage matrix) using forward variable selection to identify significant predictors of taxonomic composition for each assemblage from among both predictor sets. Finally, we performed a series of four CCAs using the two sets of significant predictors: (*i*) CCA of the assemblage matrix constrained by the LUIs, (*ii*) CCA of the assemblage matrix constrained by the OTHERs, (*iii*) like (*i*), after removing effects attributable to the OTHERs, and

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Table 2. CCA variation components and their contributing factors: variation in assemblage composition wholly attributable to LUIs, wholly attributable to OTHERs, shared among both sets of predictors, and attributable to neither set of predictors.

CCA variation component
Variation wholly attributable to LUIs
Direct effects of land use on the biota (e.g., association of riparian birds with shoreline residential development because residential areas serve as habitat for some bird species)
Effects of land use on the biota through its influence on OTHERs not analyzed (e.g., association of benthos with watershed agriculture as a result of unmeasured agricultural chemical introductions into lakes)
Variation wholly attributable to OTHERs
Responses of the biota to aspects of the environment unrelated to land use (i.e., "natural" drivers to which humans are unresponsive)
Shared variation attributable to covarying LUIs and OTHERs
Effects of land use on the biota through its effects on OTHERs (e.g., association of diatoms with covarying phosphorus and land use variables as a result of human-induced lake eutrophication)
Responses of humans and the biota to OTHERs as a result of nonanthropogenic factors (e.g., association of riparian birds with covarying climatic and land use variables because climate influences bird distributions and land use)
Variation not attributable to LUIs or OTHERs (i.e., unexplained)
Stochastic processes (e.g., immigration, extinction)
Measurement errors
Other factors not taken into account

(*iv*) like (*ii*), after removing effects attributable to the LUIs (Borcard et al. 1992). For steps (*iii*) and (*iv*), CCA analyzed the assemblage–environment variation left unaccounted for by the set of predictors whose effects were removed using multiple linear regression (ter Braak 1987*b*). The relative strengths of assemblage associations with the LUIs and OTHERs were determined using the approach of Borcard et al. (1992) (discussed below) based on results of these four CCAs.

To be chosen in the forward selection procedures, variables had to pass at a type I error probability (*P* value, determined using a 999-trial Monte Carlo test) that was readjusted at each step using the Holm correction for multiple comparisons (Holm 1979). The Holm criterion helped ensure that the probability of erroneously including one or more environmental variables in the CCA model was ≤ 0.05. It also made variable selection more stringent for the OTHERs than the LUIs. For the 26 OTHERs, the first variable had to pass at a *P* value of 0.0019 (= 0.05/26). If it was significant at that level, the second had to pass at 0.0020 (= 0.05/25), the third at 0.0021 (= 0.05/24), and so on. For the nine LUIs, the first variable had to pass at 0.0056 (= 0.05/9), the second at 0.0063 (= 0.05/8), and so on. Without the Holm correction, the partitioning of assemblage variation would be strongly biased towards the larger predictor set (in this case, the OTHERs) because of the multiple comparisons problem (D. Borcard, personal communication).

The total assemblage variation explained collectively by the LUIs and OTHERs was equal to the sum explained by steps (*i*) and (*iv*) or, equivalently, steps (*ii*) and (*iii*) from above (Borcard et al. 1992). The explained variation was partitioned as follows: (*a*) assemblage variation that was wholly attributable to the LUIs (step (*iii*)), (*b*) assemblage variation that was wholly attributable to the OTHERs (step (*iv*)), and (*c*) assemblage variation that was shared among covarying LUIs and OTHERs (step (*i*) minus step (*iii*) or, equivalently, step (*ii*) minus step (*iv*)). We expected the LUIs and OTHERs to be interrelated because land use is affected by climate and geomorphology (Omernik 1987) while affecting other aspects of the environment (e.g., increasing lake productivity). Variation partitioning allowed us to assess the extent to which assemblages were associated with covarying relationships among the two sets of predictors (Table 2).

We also partitioned the assemblage variation among environmental and spatial components in order to determine the amount of spatial structure in taxonomic composition and its correlates (Borcard et al. 1992). Significant environmental predictors of taxonomic composition were identified for each assemblage matrix using forward variable selection in CCA, this time choosing from among all 35 variables in Table 1. As before, significance was assessed using a 999-trial Monte Carlo test with a Holm-corrected *P* value. Significant spatial gradients in taxonomic composition were identified by selecting from among nine variables representing spatial location (x, y, x^2, xy, y^2, x^3, x^2y, xy^2, and y^3, where x and y are longitude and latitude coordinates for each lake) using identical techniques. Quadratic and cubic terms for the spatial coordinates and their interactions were analyzed to allow for the identification of nonlinear spatial gradients in taxonomic composition (Borcard et al. 1992). Significant environmental and spatial predictors were submitted to CCA to partition the assemblage variation among spatially structured and unstructured environmental variation and space alone using methods outlined above.

Assessing assemblage concordance

We assessed the concordance of the assemblages' dominant environmental gradients by determining correlations among site scores on the first CCA axes (CCA1) for models whose predictors were chosen from among all 35 variables in Table 1. CCA1 site scores positioned each site along the gradient and were calculated as linear combinations of environmental variables. A high correlation among CCA1 site scores would indicate that a similar suite of variables was chosen for two CCA models and that the dominant environmental gradients were similar for the assemblage pair. Significance levels are not reported for these correlations because in instances where CCA models shared one or more environmental variables, the assumption of independence was violated. All lakes with available data were used in generating the CCA model for each assemblage to maximize statistical power (from a minimum of 127 lakes for the benthos to a maximum of 186 lakes for the birds). However, only CCA1 scores for the subset of 114 lakes with data for all five assemblages were used to assess assemblage concordance. Preliminary analyses indicated that results were similar whether all available data or data for the subset of 114 lakes were analyzed.

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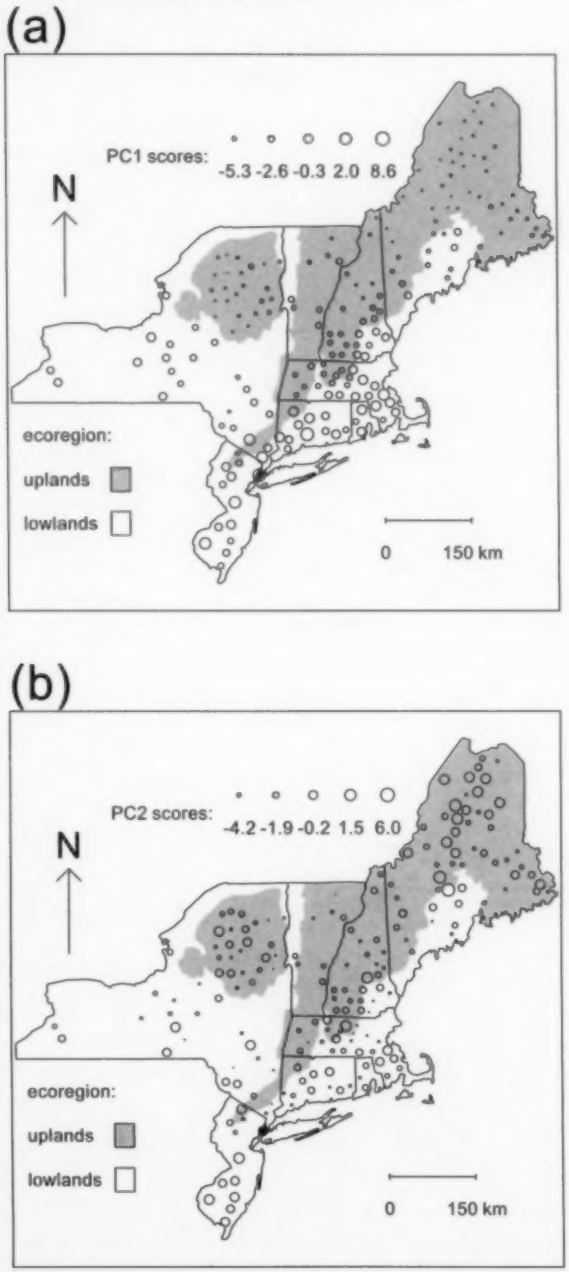
Fig. 1. Spatial distributions of principal component scores on environmental (*a*) PC1 and (*b*) PC2 for 186 northeastern U.S.A. lakes. Circle size increases continuously with the magnitude of the score. PC1 reflects differences between the upland and lowland ecoregions with respect to climate, elevation, vegetation, soil, lake productivity, and human population density; PC2 reflects more local differences related to lake morphology, productivity, and acidity. Refer to Table 1 for relationships of individual variables with the principal component axes. Locations of some lakes have been adjusted to minimize overlap.



We determined the concordance of the assemblages' dominant gradients in taxonomic composition by first subjecting data for each assemblage to detrended correspondence analysis (DCA) and then determined correlations among site scores on the first DCA axes (DCA1). In addition to characterizing gradients in taxonomic composition, DCA site scores constitute "latent" environmental gradients to which the assemblage responds that are inferred directly from the assemblage data (ter Braak 1987*a*). DCA ordinations were performed using all available data, but only DCA1 scores for the subset of 114 lakes with data for all five assemblages were used to assess concordance.

Geographic sets analyzed

The Northeast is heterogeneous with respect to broad-scale anthropogenic and nonanthropogenic factors (Omernik 1987 and results that follow). We analyzed the entire regional sample of lakes to assess the relative importance of broad-scale factors on the lake assemblages. To partition out the broadest-scale environmental heterogeneity and thus permit a better resolution of more local structuring agents, we also analyzed data for lakes in each of two ecoregions (111 upland lakes, 75 lowland lakes) (Fig. 1).

Results

Environmental gradients

The first two principal component axes captured 40% of the variance (Table 1), indicating considerable covariation among the 35 environmental variables. Environmental PC1 distinguished between relatively unproductive lakes at high elevations, in landscapes with few people, extensive conifer forests, and severe climate to the north and more productive lakes at lower elevations, in landscapes with greater human densities, deciduous forests, and milder climates to the south (Fig. 1*a*). Scores on environmental PC1 differed significantly between the upland and lowland ecoregions (one-way ANOVA, $P < 0.001$). All nine LUIs were positively correlated with environmental PC1 ($r \geq 0.37$) (Table 1). Human population density and chloride showed the strongest correlations ($r = 0.90$ and 0.85, respectively). Variables whose values increase with lake trophic state (phosphorus, turbidity, chlorophyll *a*, nitrogen) were also positively correlated with environmental PC1 ($r \geq 0.48$), as were the two deciduous forest measures ($r = 0.52$ and 0.47) and calcium ($r = 0.51$). This contrasted with negative correlations for other nonanthropogenic measures (seasonality, mixed-coniferous forest, elevation; $r \leq -0.44$).

Environmental PC2 largely reflected lake morphology and related factors judging from its negative correlations with lake depth and area and its positive correlation with minimum temperature ($r = -0.81$, -0.38, and 0.66, respectively) (Table 1), i.e., smaller, shallower lakes are generally warmer. Correlations of environmental PC2 with the lake productivity indicators (phosphorus, turbidity, chlorophyll *a*, nitrogen; $r \geq 0.45$), lakeshore wetland ($r = 0.57$), dissolved organic carbon ($r = 0.75$), and substrate size ($r = -0.56$) were also consistent with an influence of lake morphology because shallower lakes tend to have more extensive littoral zones, higher productivity, higher concentrations of dissolved organic carbon, and finer littoral sediment particle sizes. Aluminum tended to increase ($r = 0.46$) and pH tended to decrease ($r = -0.41$) with decreasing lake depth and increasing productivity on this gradient. Scores on environmental

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Table 3. Environmental correlates of assemblage composition determined using forward variable selection in CCA ($P \leq 0.05$, 999-trial Monte Carlo test, Holm-corrected P value) for lakes of the uplands (U), lowlands (L), and both (B) ecoregions combined (Fig. 1).

	Benthos			Birds			Diatoms			Fish			Zooplankton			
Variable	U	L	B	U	L	B	U	L	B	U	L	B	U	L	B	Total
LUIs																
Human population density in watershed[a]			1	1	1	1				1	1	1	1		1	9
% landscape in urban[b]		1		3		+			2					1		4
% watershed in agriculture[c]	2				2	+	1		1							4
% shoreline in residential[d]				2	3	2										3
% landscape in agriculture[b]				+	+	+				2		2				2
Road density in watershed[a]									3	3						2
% watershed in urban[c]			2			3										2
% shoreline in agriculture[d]						+									2	1
Watershed point-source pollution[e]	1															1
OTHERs																
Seasonality (July–Jan. mean temperature)[b]			1	+	2	2	+	3	3	1	3	1	3			9
Maximum lake depth[d]						+	2	2	2		1	2		3	+	6
pH[d]							1	1	1				1	2	1	6
Calcium[d]	2		2		3	+	3		+		2	3	+		+	6
Chloride[d]				1	1	1									3	4
Phosphorus[d]		2	3					+		+						2
% shoreline in wetland[d]				3		3										2
% landscape in deciduous forest[b]	1			2		+									+	2
Turbidity[d]			+											1	2	2
Lake area							+		+	2		+			+	1
% shoreline in conifer forest[d]		1		+		+						+				1
Sulfate[d]										3						1
Minimum water temperature[d]													2			1
% shoreline in deciduous forest[d]				+	+	+			+						+	0
Elevation				+		+				+		+				0
Nitrogen[d]				+			+		+							0
Aluminum[d]						+			+						+	0
Chlorophyll a[d]							+		+						+	0
% shoreline in mixed forest[d]				+		+										0
Dissolved organic carbon[d]									+							0
Macrophyte cover[d]						+										0
Silica[d]									+							0
% landscape in mixed-conifer forest[b]																0
Substrate size[d]																0
Minimum dissolved oxygen[d]																0
% watershed in wetland[c]																0

Note: LUIs and OTHERs were analyzed separately. The first three variables entering the models (1, 2, 3) are distinguished from subsequent additions (+). Row totals are equal to the number of models where the variable was among the first three chosen. Data sources or collection methods are footnoted.

[a] U.S. Bureau of Census (1990).
[b] O'Connor et al. (1996).
[c] Anderson et al. (1976).
[d] Baker et al. (1997).
[e] Abramovitz et al. (1990).

PC2 did not differ significantly between the two ecoregions (one-way ANOVA, $P = 0.81$) (Fig. 1*b*).

Assemblage–environment associations

Significant LUIs were identified in all forward selection procedures except one, diatom assemblages in the lowlands (Table 3), where the first LUI to enter the model (road density in the watershed; $P = 0.0060$) was just below the significance threshold required to meet the Holm criterion ($P = 0.0056$). Overall, human population density was the most important LUI, selected first nine out of 15 times. Significant OTHERs were identified in all 15 forward selection procedures (Table 3), which are summarized as follows.

Benthic macroinvertebrates

Landscape deciduous forest, shoreline coniferous forest, and seasonality were the first variables to enter benthic macroinvertebrate models for lakes of the uplands, lowlands,

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and the entire region, respectively. The composition of benthic macroinvertebrates was thus most closely associated with forest type and climate. Subsequent variable additions indicated water quality influences (calcium, phosphorus).

Riparian birds

Chloride was selected first in all three riparian bird models. Subsequent variable additions included seasonality, landscape deciduous forest, lakeshore wetland, and calcium.

Sedimentary diatoms

pH and lake depth were identified as being the two most significant correlates of diatom composition for all three geographic sets. Subsequent variable additions indicated influences related to climate (seasonality) and ionic strength (calcium).

Fish

Seasonality was most important for the fish in two of the three models. Other variable additions indicated influences of lake morphology (area, depth) and water chemistry (calcium, sulfate).

Pelagic zooplankton

As with the diatoms, the zooplankton showed their strongest correlations with pH. Zooplankton composition was also correlated with factors related to lake trophic condition and morphology (turbidity, lake depth, minimum water temperature) and with chloride and seasonality.

Partitioning assemblage variation among predictors

The amount of assemblage variation explained collectively by the LUIs and the OTHERs in Table 3 ranged from 10 to 31%. The goal of CCA is to predict synoptic patterns of assemblage composition rather than distributions of individual taxa, so CCA models that explain low proportions of the total assemblage variation can be ecologically informative (ter Braak 1987*a*). For this study, we were most interested in the explained variation. Results that follow have therefore been expressed as proportions of the explained assemblage variation attributable to different sets of predictors.

Assemblage variation in some way attributable to land use (wholly LUI variation plus shared LUI–OTHER variation) was proportionally as great or greater for the benthos, birds, and fish as for the other assemblages regardless of the geographic set (Fig. 2*a*). The larger-bodied assemblages thus showed a closer correspondence to land use than did the diatoms and zooplankton. The shared LUI–OTHER variation was consistently greater for the region as a whole than for either ecoregion, indicating that anthropogenic and nonanthropogenic correlates of taxonomic composition became more confounded at broader spatial extents.

Assemblage variation in some way attributable to space (wholly spatial plus shared environmental–spatial variation) was greater for the benthos, birds, and fish than for the other assemblages in the uplands and in the region as a whole (Fig. 2*b*), a pattern largely attributable to the shared environmental–spatial components. The larger-bodied assemblages therefore demonstrated more spatial structure with respect to site-to-site variation in taxonomic composition. In the lowlands, the magnitudes of variation components showed no differences between the larger- and smaller-bodied assemblages.

Fig. 2. Percentages of explained assemblage variation attributable to (*a*) LUIs and OTHERs and (*b*) environmental variables (LUIs and OTHERs combined from Table 1) and variables representing spatial location for the five assemblages in lakes of the uplands, lowlands, and both ecoregions combined (Fig. 1). Spatial variables included longitude, latitude, and seven derived variables representing quadratic and cubic terms and their interactions. Significant predictors were independently chosen from among each of the four predictor sets (LUIs, OTHERs, environmental and spatial variables) using forward variable selection in CCA ($P \leq 0.05$, 999-trial Monte Carlo test, Holm-corrected P value). Assemblage variation was partitioned among variables in CCA models using the approach of Borcard et al. (1992).

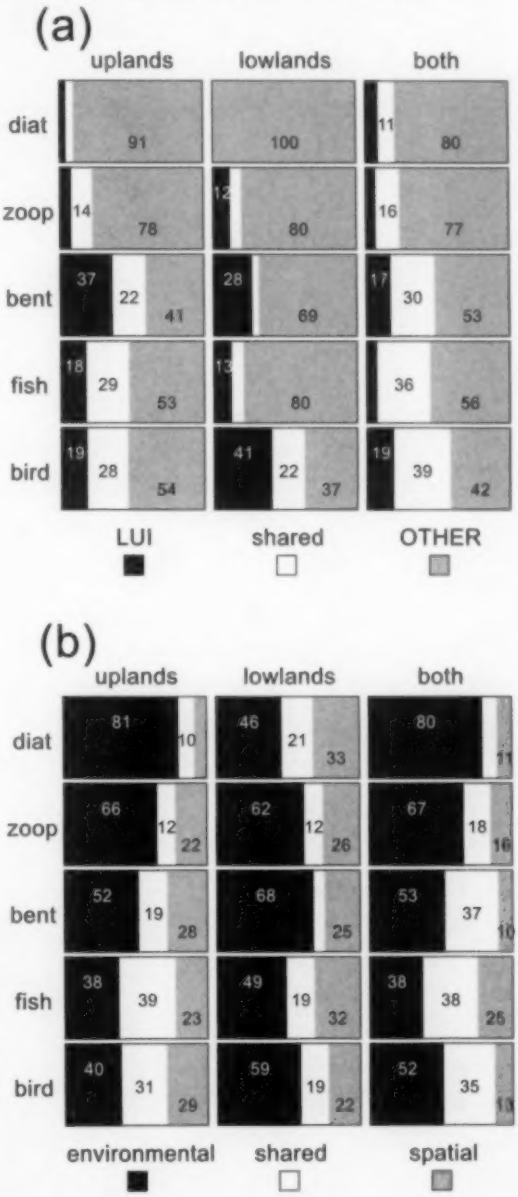

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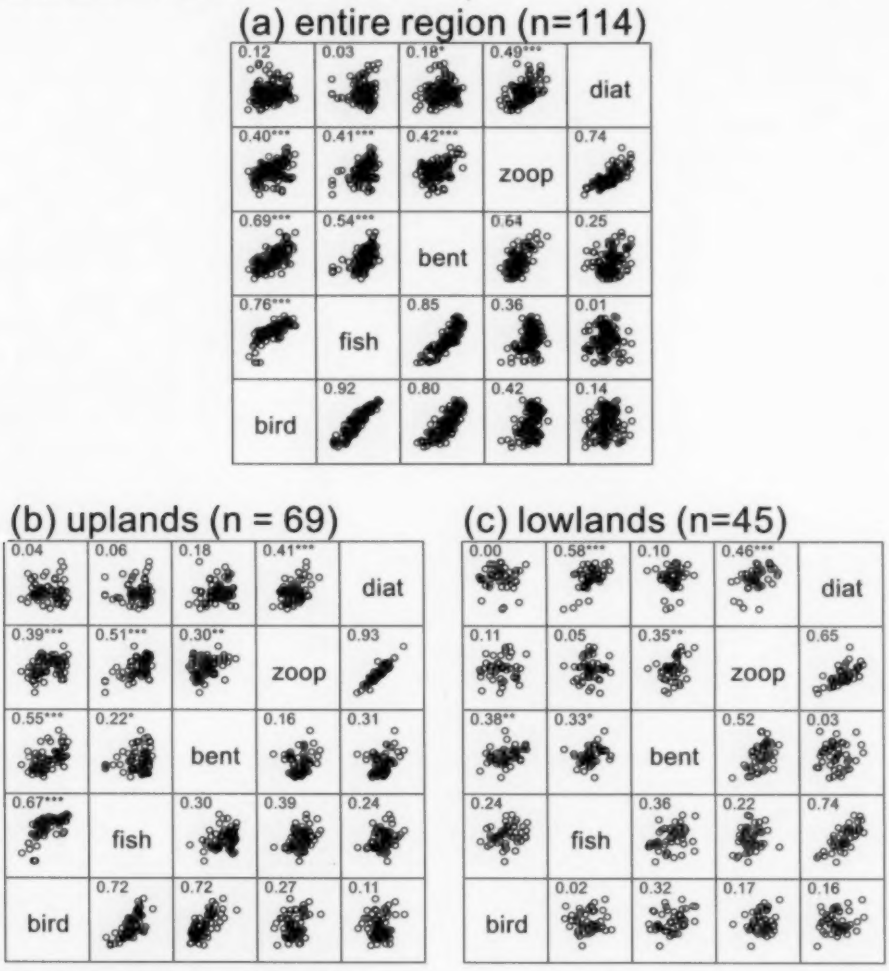
Fig. 3. Scatterplot matrices and Pearson correlations (r) among site scores on DCA1 (above the diagonal) and CCA1 (below the diagonal) for ordinations of data from (*a*) lakes throughout the region and from lakes restricted to the (*b*) upland and (*c*) lowland ecoregions (Fig. 1). All available data were used to perform ordinations for each assemblage, but only scores for the 114 lakes with data for all five assemblages are presented here. Significance levels are reported for DCA1 correlations (one-tailed test) but not for CCA1 correlations because the assumption of independence was violated in instances where CCA models shared environmental variables. Environmental variables were selected for CCA models from among all 35 variables in Table 1 using forward variable selection ($P \leq 0.05$, 999-trial Monte Carlo test, Holm-corrected P value).



Assemblage concordance

Comparisons among CCA1 site scores for models incorporating significant predictors of taxonomic composition (chosen from among all 35 variables in Table 1) are presented below the diagonals of scatterplot matrices for the five assemblages (Fig. 3). The variables selected for these models tended to be subsets of those selected in Table 3 and are not presented.

For the region as a whole, as differences in body size increased, the concordance of the assemblages' dominant environmental gradients decreased (Fig. 3*a*, below diagonal). For example, CCA1 site scores for the birds showed stronger correlations with those for the fish (r = 0.92) than with those for the benthic macroinvertebrates (r = 0.80), zooplankton (r = 0.42), or diatoms (r = 0.14). Correlations among DCA1 site scores tended to be lower than among CCA1 site scores, but concordance was still generally higher for assemblages whose taxa were more similar in body size (Fig. 3*a*, above diagonal). Assemblage concordance showed a weaker or nonexistent relationship to body size in the upland and lowland ecoregions (Figs. 3*b* and 3*c*, respectively).

Discussion

Environmental gradients

The high correlation of human population density with

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this region's dominant environmental gradient (environmental PC1) reflected the constraints imposed by climate and geomorphology on land use (Omernik 1987) and, in turn, the influence of land use on other aspects of the environment. The climate and soils of northern New England and the Adirondacks of northeastern New York are better suited to forestry than to agriculture and other intensive land uses. Private ownership of land in northern Maine by paper and timber companies and public ownership of land in the Adirondacks have served to limit human densities in these areas as compared with the rest of the region (Whittier et al. 1997). Climatic and geomorphological influences on land use thus explain the close correspondence of land use measures to climate, forest composition, elevation, and calcium on environmental PC1.

Land use has, in turn, introduced nutrients, chloride, and other chemicals into lakes of the Northeast (Dixit et al. 1999), linking lakes to broader-scale human systems (Carpenter and Cottingham 1997) and increasing the correspondence between the LUIs and some of the OTHERs on environmental PC1. Chloride showed a close correspondence to human population density ($r = 0.86$) and environmental PC1 because human activities (e.g., road deicing) have increased lake chloride concentrations in the region (Dixit et al. 1999). The lake productivity indicators showed a positive correlation with environmental PC1, in part because lakes to the south tended to be shallower, smaller, and more calcium rich on this gradient (Table 1). Paleolimnological evidence indicates that anthropogenic lake eutrophication has also been a contributing factor because, in contrast eith other lakes of the region, the productivity of many urbanized lakes in the Northeast has increased since preindustrial times (Dixit et al. 1999).

Superimposed on the regional environmental gradient were lake morphological effects, which induced local-scale differences between lakes with respect to trophic status, riparian – littoral zone structure, and acidity on environmental PC2. The lake productivity indicators generally showed stronger correlations with environmental PC2 than with environmental PC1 (Table 1), indicating that local effects of lake morphology were at least as important as regional factors in determining the trophic status of individual lakes.

Assemblage–environment associations

The selection of human population density as the most important LUI in most forward selection procedures likely reflected a combination of anthropogenic and nonanthropogenic factors. This is because human population density showed a close correspondence to this region's dominant environmental gradient as a result of both human effects on the environment and human responses to the environment. The failure to detect a significant association between lowland diatom composition and the LUIs may have been a type II error because diatom assemblages are sensitive to phosphorus, chloride, and other chemicals introduced into lakes by human activities (Dixit et al. 1999). Alternatively, anthropogenic effects on lowland diatom assemblages may have been relatively homogeneous, offering little gradient for detection.

Selecting exclusively from among the OTHERs, the larger-bodied assemblages (benthos, birds, fish) generally showed their strongest associations with variables that exhibited broad-scale spatial structure on environmental PC1 (chloride, seasonality, forest composition). Chloride likely served as a surrogate for regional patterns of land use and their correlates in the three riparian bird models because (*i*) chloride was well correlated with human population density, the most important LUI for the birds, (*ii*) chloride showed a close correspondence to environmental PC1 as a result of land use, and (*iii*) the lakeshore bird assemblages comprised predominantly terrestrial passerines (~80% of all individuals surveyed), a group sensitive to fragmentation of forests by land use (Allen and O'Connor 1999). The selection of seasonality as the first OTHER in the regional and upland fish models, followed by lake depth and water chemistry variables, is consistent with the hypothesis that climate and postglacial dispersal barriers dictate fish distributions at broad spatial scales but that lake morphology and water chemistry determine the species present in individual lakes (Jackson and Harvey 1989; Tonn 1990).

In contrast, the diatoms and zooplankton showed their strongest correlations with OTHERs exhibiting local-scale variation on environmental PC2 (pH, lake depth). The sensitivity of diatom assemblages to the water chemistry is well documented (e.g., Dixit et al. 1999). For a subset of the lakes analyzed here, Stemberger and Lazorchak (1994) identified not only abiotic factors as important determinants of zooplankton composition, but also fish trophic structural variables. The associations that we observed may therefore reflect a combination of direct environmental effects and environmentally mediated biotic effects, e.g., turbid waters offering zooplankton visual refuge from fish predation.

Partitioning assemblage variation among predictors

Environmental–spatial partitioning

For the region as a whole and, to a lesser extent, in the uplands, the proportions of assemblage variation attributable to the shared environmental–spatial components were greater for the larger-bodied assemblages than for the diatoms and zooplankton. These findings are consistent with the larger-bodied assemblages being more strongly correlated with variables exhibiting broad-scale spatial structure on environmental PC1.

As much as a third of the explained assemblage variation was wholly attributable to space when partitioning assemblage variation among environmental and spatial predictors (Fig. 2*b*). The environmental variables analyzed here therefore could not account for all spatially structured variation in taxonomic composition. The wholly spatial variation may reflect one or more of the following: responses of assemblages to unmeasured, spatially structured environmental variables, the effects of spatially mediated ecological processes (e.g., dispersal) on assemblage composition, or the effects of historical events such as glaciation on taxon distributions (Borcard et al. 1992).

LUI–OTHER partitioning

The larger-bodied assemblages (benthos, birds, fish) showed a closer correspondence to land use than did the diatoms and zooplankton. Our results indicate that the dominant

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nonanthropogenic factors affecting land use and distributions of the larger-bodied taxa were similar (climate, geomorphology) and of broader scale than those most important to the diatoms and zooplankton (pH, lake depth). Hence, even if anthropogenic factors had no effect on the biota of this region, we would expect the larger-bodied assemblages to show a closer correspondence to land use.

We argue, however, that human activities have had greater and more direct effects on the larger-bodied assemblages examined, thereby contributing to their greater correspondence to land use. The terrestrial habitat use of riparian birds makes them responsive to incremental changes in land use locally (Croonquist and Brooks 1991) and regionally (Allen and O'Connor 1999). Nonnative fish introductions, along with concomitant effects on the native piscifauna (e.g., extirpation of native minnows), have increased the correspondence between fish distributions and land use at broad spatial scales (Whittier et al. 1997). These introductions affect other assemblages (e.g., zooplankton, Stemberger and Lazorchak 1994), but the magnitudes of their effects are dependent on characteristics of individual lakes (Li and Moyle 1981). Human introductions of other organisms have also occurred (e.g., zooplankton, Stemberger 1995; zebra mussels, Whittier et al. 1995), but these have been less frequent and directed than for the fish.

Humans have also restructured lake biota of this region by introducing chemicals into lakes (Stemberger and Lazorchak 1994; Dixit et al. 1999). However, multiple factors mediate a lake's susceptibility to this mode of anthropogenic influence (Carpenter and Cottingham 1997). This is consistent with our PCA of the environmental data, which showed lake productivity to be more closely associated with lake depth than with land use despite paleoecological evidence indicating that anthropogenic lake eutrophication has occurred across much of the lowlands (Dixit et al. 1999).

Much of the explained assemblage variation was shared among covarying LUIs and OTHERs, particularly for larger-bodied assemblages (Fig. 2*a*). Evaluation of LUIs or OTHERs alone may therefore lead one to overestimate effects attributable to either set of predictors (by some portion of the shared variation), potentially leading to erroneous conclusions about the mechanisms involved (Allan and Johnson 1997). Even when both sets of predictors are analyzed, the shared variation presents challenges to interpretation (Table 2). For example, the shared variation may have increased with spatial extent for all five assemblages because anthropogenic effects on the biota occurred more regionally than locally (e.g., forest fragmentation effects on the avifauna, Allen and O'Connor 1999; effects of fish species introductions on the piscifauna, Whittier et al. 1997; Whittier and Kincaid 1999). It may have also increased, however, because the nonanthropogenic factors integrated by humans and other organisms increased in similarity at broader extents as climate and geomorphology assumed greater importance.

Identifying that portion of the shared variation attributable to humans requires extending the analysis into the temporal dimension. Dixit et al. (1999) circumvented the problem of confounded anthropogenic and nonanthropogenic effects by using paleoecological diatom data to infer that the relatively high trophic status of lakes in southern New England was partly attributable to anthropogenic eutrophication. Where antecedent data are unavailable, the only recourse is to infer process from pattern based on ecological understanding. Anthropogenic and nonanthropogenic correlates of fish distributions were largely confounded regionally (Fig. 2*a*). Despite this, Whittier et al. (1997) argued that regional minnow biodiversity losses have occurred in the Northeast as a result of game fish introductions because contemporary data are consistent with this mode of anthropogenic influence and because these effects have been documented in other lake districts (e.g., Chapleau et al. 1997). Human-induced environmental change is also assessed by comparing sites assumed to have little or no overt human influence (i.e., reference sites) with other areas (Karr 1991). Our results suggest that this approach should be applied with caution at the regional scale because "reference sites" of the Northeast differed from other areas with respect to broad-scale nonanthropogenic factors that presumably rendered them less suitable to intensive land uses, thereby confounding inferences regarding anthropogenic environmental change.

Assemblage concordance

Our results indicate that the larger-bodied assemblages were associated more closely with broad-scale factors than were the diatoms and zooplankton. The observed patterns of concordance therefore appear to reflect differences in the scales at which the larger- and smaller-bodied assemblages integrated the environment. They are not an artifact of the environmental variables analyzed because results were qualitatively similar whether analyses were undertaken using CCA or DCA. The similarity of the CCA and DCA results for the region as a whole indicates that the environmental variables included in the CCA models were sufficient to generate the observed patterns of concordance. It appears, therefore, that biological interactions among these assemblages need not be invoked to explain the patterns. This is not surprising given the broad extent of this study because the importance of biotic factors is thought to decrease with increases in spatial scale (Tonn 1990).

The diatoms and zooplankton have less complex physiological, morphological, and behavioral adaptations than the fish and higher surface to volume ratios, rendering them more sensitive to the water chemistry and other local-scale details of the environment. The diatoms and zooplankton also respond more rapidly to environmental change than the fish because of their shorter life spans and greater powers of dispersal among lakes (Schindler 1987). For example, Stemberger et al. (1996) showed that unusually cool temperatures in 1992 coincided with increases in zooplankton richness for small cladocerans and rotifers but not for large cladocerans and copepods. The temperature signal thus appeared to be of sufficient duration and magnitude to affect small zooplankton but not large zooplankton. This climatic event also had no significant effect on the richness of fish assemblages in the Northeast (A.P. Allen, unpublished data), as one would expect given that water temperatures were only 1–2°C below normal for a single year (Tonn 1990).

Longer-term climatic variability, on the other hand, would be expected to have greater effect on the fish than on the smaller-bodied assemblages. Fish species will require longer periods of time than diatoms and zooplankton to recolonize

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isolated lakes after climatically driven events (e.g., low dissolved oxygen incursions) because of their relatively low dispersal capabilities and reproductive potential. We would thus expect the fish to show a closer correspondence to climatic patterns than the diatoms and zooplankton. The limited dispersal capabilities of the fish also makes them particularly sensitive to historical events such as glaciation (Jackson and Harvey 1989) and nonnative species introduction (Whittier et al. 1997). Despite having extensive dispersal capabilities, the riparian birds integrated the environment at a scale similar to that of the fish. Many bird species are sensitive to forest composition and the size of contiguous forest tracts, both of which are functions of climate, geomorphology, and land use (Allen and O'Connor 1999).

Local-scale factors will necessarily assume greater importance over smaller areas as the range of variation in climate and geomorphology is reduced (e.g., Lammert and Allan 1999), thereby obscuring habitat scaling differences among assemblages. This explains why assemblage concordance showed a weaker relationship to body size for the two ecoregions as compared with the region as a whole. For example, the fish and diatoms showed no significant association in DCA1 site scores for the region as a whole but a significant relationship in the lowland ecoregion Fig. 3). This would occur if climatic factors were of far greater importance to fish than to diatoms regionally owing to the broader scale at which fish integrate the environment, and if factors related to lake depth were important to both assemblages locally.

Conclusions

We observed a high degree of broad-scale covariation among anthropogenic and nonanthropogenic variables affecting lake biota of the northeastern U.S.A., reflecting both human alterations of the environment and human responses to the environment. These results emphasize the importance of analyzing interrelationships among anthropogenic and nonanthropogenic variables as part of ecological studies at the regional scale. They also emphasize the importance of long-term environmental monitoring, paleoecological analysis, and other approaches that give explicit consideration to environmental change through time to disentangle broad-scale anthropogenic and nonanthropogenic effects.

The observed patterns of concordance indicate that these assemblages responded to the environment at different scales. Moreover, concordance patterns were themselves scale dependent, resulting at least in part from scale dependencies in the environmental heterogeneity to which these assemblages responded. Differences in habitat scaling among assemblages showed a relationship with body size. Body size imposes fundamental constraints on physiological parameters affecting habitat scaling such as life span, metabolic rate, and reproductive potential (Peters 1983), but for our assemblages, body size was confounded with vast differences in taxonomy. It thus appears that multiple factors, some of which are related to body size for the aquatic taxa (e.g., niche breadth, dispersal capability), determined the habitat scaling differences that we observed.

The covariation among anthropogenic and nonanthropogenic factors, the varied responses of assemblages to these factors, and the scale dependence of assemblage responses suggest two important implications for conservation. First, relationships that exist between land use and other factors may result in the systematic elimination of habitat for species that respond to the environment at scales similar to humans (e.g., migratory bird species occupying deciduous forests, Allen and O'Connor 1999; lentic minnows occupying low-elevation lakes, Whittier et al. 1997). Second, monitoring a single assemblage, let alone a single species, may only be useful for detecting changes in particular aspects of the environment and only over a limited range of scales. Indicator taxa should therefore be chosen only after careful consideration of the types of changes one hopes to detect and the scales over which one hopes to do so.

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Interplay between dispersal determinants of larval hydropsychid caddisflies

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Abstract: We examined the interactive effects of predator (perlid stoneflies) presence, epilithic algal cover, and substrate size on drift and resettlement decisions by larval hydropsychid caddisflies in a series of experiments conducted in real and artificial streams. Predator effects on drift-entry depended on algal structural complexity. Stoneflies significantly increased hydropsychid drift from biofilm-covered rocks but not from rocks covered with a thick periphyton mat or with a macroalga (*Cladophora glomerata*, ~20% surface cover). When we manipulated degree of *Cladophora* cover, we found that *Cladophora* even in small amounts (4–13% cover) nullified stonefly effects on drift and that drift was inversely related to amount of *Cladophora* on rocks. However, *Cladophora* may only be important as a dispersal determinant until retreats (i.e., immovable cases) are constructed and vulnerability to predators decreases. Hydropsychid drift rates were positively related to gravel abundance, but *Cladophora* presence enhanced settlement rates on the substrate. Our study shows that multiple factors jointly influence dispersal patterns of hydropsychid larvae and that the relative strength of any one factor as a dispersal determinant varies depending on conditions of other determinants.

Résumé : Nous avons examiné les effets interactifs de la présence de prédateurs (perlidés), du couvert algal épilithique et du type de substrat sur la dérive et le dépôt des larves d'hydropsychidés dans le cadre d'expériences effectuées en cours d'eau naturels et artificiels. Les effets des prédateurs sur l'entrée en dérive dépendaient de la complexité structurale du couvert algal. Les perlidés accroissaient significativement la dérive des hydropsychidés déposés sur des roches couvertes d'un film biologique mais pas celle de ceux déposés sur des roches couvertes d'un épais tapis de périphyton ou d'une grande algue (*Cladophora glomerata*, couverture d'environ 20%). Quand nous avons joué sur la couverture de *Cladophora*, nous avons observé que, même faible (4–13%), elle annulait les effets des perlidés sur la dérive et que cette dernière variait en raison inverse de la quantité de *Cladophora* sur les roches. Cependant, les cladophores pourraient ne jouer un rôle important dans la dispersion des larves que jusqu'au moment où des abris (c'est-à-dire des fourreaux fixes) sont construits et que la vulnérabilité aux prédateurs diminue. Les taux de dérive des hydropshychidés variaient en raison directe de l'abondance de gravier, mais la présence des cladophores augmentait les taux de dépôt sur le substrat. Notre étude montre que divers facteurs influent ensemble sur les profils de dispersion des larves d'hydropsychidés et que l'impact relatif de l'un ou l'autre de ces facteurs sur la dispersion dépend des autres facteurs.

[Traduit par la Rédaction]

Introduction

Dispersal movements can profoundly affect the population dynamics, local distributions, and strengths of social and species interactions of mobile species. Both empirical and theoretical evidence suggests that environmental variation plays a key role in determining dispersal decisions (reviewed by Johnson and Gaines 1990; Mackay 1992). Spatiotemporal heterogeneity in environments creates habitat patches that differ in suitability because of differences in microhabitat physical characteristics, resource profitability, and (or) predation risk. However, the relative influence of these factors on movement decisions is often difficult to assess because these factors interact and the relative strength of a factor varies at different times or places. While some studies have examined how multiple factors concurrently affect dispersal movements of aquatic organisms, including fishes (Schlosser 1995), amphibians (Gibbs 1998), insects (Fonesca and Hart

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1996), isopods (Holomuzki and Short 1988), and molluscs (Crowe 1999), our knowledge of how the relative strengths of these factors change with ever-changing environmental conditions is poor.

Here, we examine proximate causes of dispersal by larval hydropsychid caddisflies. Like many other lotic macroinvertebrates, these organisms disperse instream by drifting; individuals are transported downstream by water flow. Larval hydropsychids are net-spinning filter-feeders, often abundant in smaller streams where they are an integral part of the food web (Mackay and Waters 1986; Pan and Lowe 1995). Many species construct retreats (i.e., immovable cases) of tiny stones or plant material on the tops of rocks in relatively swift currents (Malas and Wallace 1977). Retreats serve a dual role as a refugium from predators (Fuller and Rand 1990) and as a substrate to attach a food-capturing net. The silken net, spun near or within the mouth of the retreat, collects small algae (e.g., diatoms), detrital seston, and small drifting animals (Lamberti and Moore 1984; Loudon and Alstad 1990). Individuals may leave the retreat to drift and construct a new one upon resettlement in a suitable benthic patch. A variety of factors seem to influence hydropsychid drift. Abiotic factors include substrate availability and texture (Benke et al. 1991; Kerans 1996) and flow patterns (Smith-Cuffney and Wallace 1987; Williams 1990), whereas biotic factors include predation risk (Michael and Culver 1987), food availability (Hildebrand 1974; Williams and Levens 1988), intraspecific aggressive encounters (Glass and Bovbjerg 1969), and life stage (developmental) changes (Kerans 1992). What remains uncertain is when and where each of these factors assumes importance and how these factors interact.

Specifically, we evaluate the interacting effects of predator (stonefly) presence, epilithic algal cover, and substrate size on instream dispersal of hydropsychids. Variation in rock size, and amount and kind of algal cover, create spatial heterogeneity, which in turn can affect predation risk. While numerous studies have documented that increased spatial heterogeneity can reduce the capacity of predators to detect or pursue their prey (e.g., Lima and Dill 1990), few studies have evaluated how multiple habitat characteristics interact to affect predator-induced dispersal decisions (Holomuzki and Short 1988; Rader 1997). We combine correlative observations from field enclosures with data from small-scale experiments to assess the joint impact of multiple factors on both drift-entry and benthic resettlement.

Materials and methods

Study system and species

The study was conducted along stretches of the East and Main branches of the Maple River and at the University of Michigan Experimental Stream Facility, located ~3 km east of Pellston, Emmit County, in northern Michigan (Fig. 1). The Stream Facility draws water from the East Branch, a warm-water, second-order stream that drains Douglas Lake. The East Branch joins the West Branch at Lake Kathleen (45.25°N, 84.45°W) to form the Main Branch. Both streams are permanent and flow through mixed conifer and birch–aspen forest. Streambed surfaces of both rivers are mostly sand (>75%), interspersed with shallow surface patches of gravel to cobble substrates underlain by sand. The epilithic algae are dominated by the small, prostrate diatoms *Cocconeis* spp., *Achnanthes amphora*, and *Fragillaria* spp. (Stevenson and Hashim 1989). Standing crop of epilithic algae is generally low because of intense grazing mainly from baetid mayflies and glossosomatid caddisflies (Kohler and Wiley 1997). The macroalga *Cladophora glomerata* is scarce in both streams but is occasionally found in patches characterized by open canopy, stable substrates, and fast currents (J.R. Holomuzki, personal observation).

Hydropsychids and *Glossosoma nigrior* (Glossosomatidae) are the dominant caddisflies in the system. The hydropsychid assemblage comprises many species, although *Ceratopsyche sparna*, *Ceratopsyche slossonae*, *Ceratopsyche morosa*, and *Hydropsyche betteni* are the most common. Fourth and fifth larval instars of both *Ceratopsyche sparna* and *Ceratopsyche slossonae* are abundant through June to mid-August. The perlid stoneflies *Acroneuria abnormis*, *Agnetina capitata*, and *Paragnetina media* are common invertebrate predators (Holomuzki 1996). *Paragnetina* emerges in late June to early July; however, *Acroneuria abnormis* and *Agnetina capitata* are present through July.

Experimental investigation of dispersal factors

Four different experiments were designed to test the interactive effects of perlid presence, epilithic algal cover, and rock size on drift-entry and benthic resettlement by larval hydropsychids. Experiments 1–3 deal with drift-entry, and experiment 4 addresses resettlement. Specific goals and methodologies differed for each experiment (Table 1).

Experiment 1. Drift from instream enclosures

We compared nocturnal drift rates of hydropsychid larvae among enclosed sections of streambed with varying physical characteristics, algal cover, and stonefly densities. Three enclosures were constructed on each of six dates (26 and 30 July and 1, 5, 9, and 13 August 1996) in different riffles in the East and Main branches of the Maple River (Fig. 1). Sample sites were selected on the basis of accessibility and the presence of a hydropsychid assemblage and *Cladophora*. Dense epilithic vegetation, such as macroalgae (e.g., *Cladophora*) and moss, used as attachment substrates for retreats and capture nets is believed to be a key determinant of hydropsychid dispersal (Dudley et al. 1986). Each of the 18 enclosures (each 0.75 m^2) consisted of two sides of solid aluminum sheeting supported by rebar, with upstream and downstream ends blocked by drift nets. Sides of enclosures (1.63 m long, 0.91 m high) paralleled the current and were sunk 5–10 cm into the substratum to prevent animals from entering or escaping. To minimize substrate disturbance, areas to be enclosed were not trampled during construction, and drift nets were not placed at the ends until ~24 h after construction of the sides. Drift nets (mouth 46 × 35 cm, 363-μm mesh) were placed at upstream and downstream ends at sunset (~21:00) to capture all animals entering and leaving the enclosed area. Current velocity, as measured with a Marsh–McBirney meter 2 cm from the bottom, was measured in enclosed substrates to assess its effect on drift. Inflow nets at the head of each enclosure gradually accumulated fine particulate organic matter (FPOM), which reduced current velocity (~15–20%) in enclosures. Downstream nets did not clog over the 12-h sampling period.

All animals were removed from nets at 09:00 and immediately preserved in 70% ethanol. Only nocturnal drift was monitored because hydropsychid larvae drift predominantly at night (Bergey and Ward 1989; Kerans 1996). We estimated percent *Cladophora* cover, percent composition of substrate particle sizes (i.e., gravels), and densities of hydropsychids and perlid stoneflies within enclosures after nets were removed. *Cladophora* cover and substrate sizes were estimated visually in each enclosure by two or more individuals. These estimates were averaged and analyzed as one observation. Hydropsychid and perlid densities were estimated from

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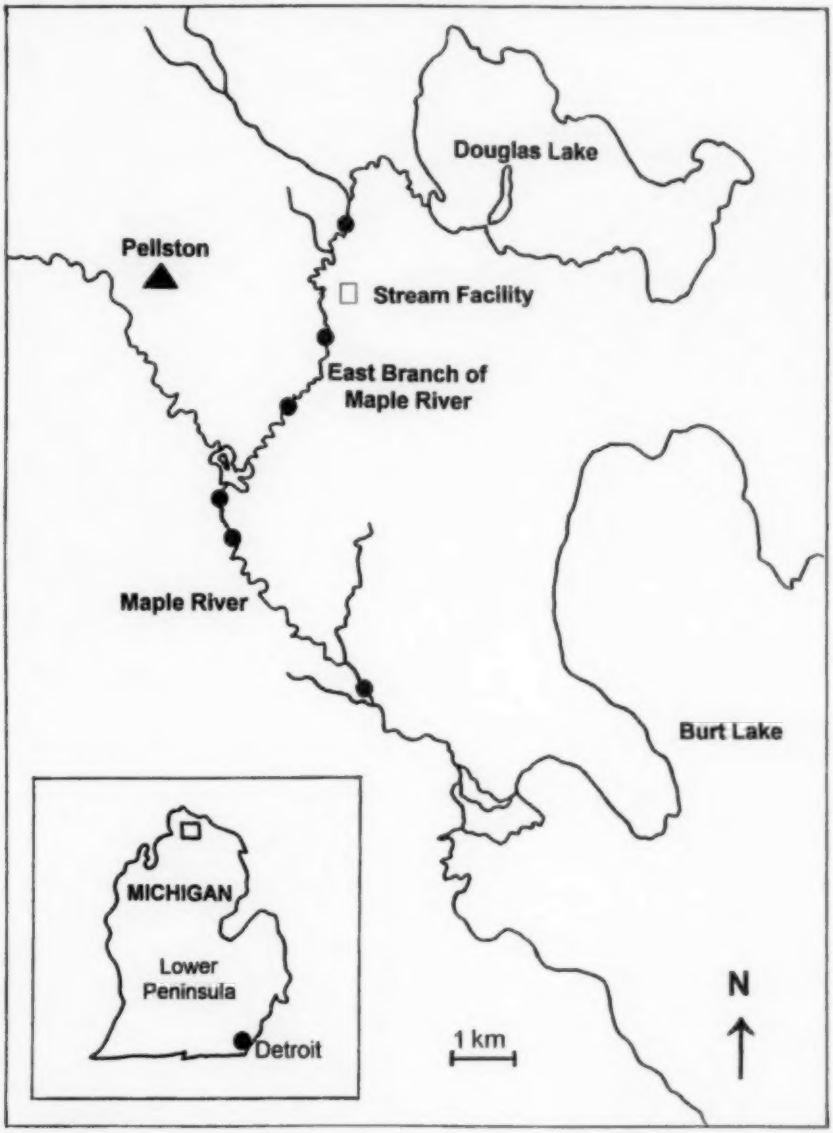
Fig. 1. Location of the University of Michigan Experimental Stream Facility and the study sites (circles) of the enclosure experiment. Inset map at the lower left shows the locality of the Main and East branches of the Maple River in Michigan.



two nonoverlapping samples per enclosure collected from 0.4 × 0.4 m quadrats outlined by wire. All surface rocks (≥1 cm in diameter, ~4 cm depth) within a quadrat were lifted into enamel pans, and then the organisms on all rocks were counted. Surber samplers (363-μm-mesh net) placed 1–5 cm downstream of each sampling quadrat captured animals separated from rocks as they were lifted from the water. Counts from substrate samples and nets from both quadrats were averaged and treated as one observation in analyses. Calculating densities enabled us to estimate the total number of study organisms in enclosures and hence compute percent drift from each enclosure. Enclosures were fish free, so the physical presence of fish on hydropsychid drift was not evaluated.

Multiple regression analysis (SYSTAT version 7.0) was used to determine the effects of *Cladophora* cover, substrate type (i.e., gravel), and perlid densities on hydropsychid drift. We had considered including current velocity and hydropsychid density in the model; however, these two independent variables were deleted to remedy multicolinearity problems. Percent *Cladophora* and percent gravels were arcsine transformed, whereas density was $\log(x + 1)$ transformed prior to analysis to homogenize variances. Transformed data here, and in other experiments, were homoscedastic according to F_{max} or Bartlett's tests.

Experiment 2. Epilithic algae–stonefly interactions

The joint influence of amount and kind of algal cover and stonefly predators on hydropsychid drift was evaluated in a two-factor factorial design experiment conducted in artificial stream channels at the Stream Facility. We measured nocturnal drift rates from sim-

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Table 1. Objectives, locations, and methodologies of the four experiments conducted to evaluate dispersal determinants of hydropsychid larvae.

Experiment	Question	Factors analyzed	Location and methods	Species
Drift-entry				
1	What factors affect larval drift in real streams in northern Michigan?	*Cladophora* cover, substrate type, perlid density	Riffles in the Main and tributary branches of the Maple River, field enclosures, 15 replicates on six dates	Assorted
2	How do epilithic algae and stonefly presence interact to affect larval drift?	Algal amount and type (biofilm, thick periphyton mat, or *Cladophora*), presence or absence of stoneflies	Stream Facility, artificial stream channels (vinyl gutters), six treatment combinations each replicated six times on one date	~70% *Ceratopsyche sparna*
3	How does degree of *Cladophora* cover affect predator-induced drift?	Epilithic *Cladophora* cover (low (4–13%), medium (20–65%), or high (80–95%)), presence or absence of stoneflies	Stream Facility, artificial stream channels (wooden), six treatment combinations replicated on seven different dates	~80% *Ceratopsyche slossonae*
Resettlement				
4	How do *Cladophora* cover and rock size interact to affect larval settlement?	Algal cover (biofilm or *Cladophora*), rock size (gravel or cobble)	Riffle in Maple River, aluminum trays, four treatment combinations each replicated 10 times, 7-day colonization period	>90% *Ceratopsyche sparna*

ilarly sized stones covered with either a thin biofilm (mostly prostrate diatoms), a thick periphyton mat (mostly stalked diatoms (i.e., *Cymbella* and *Gomphonema*) with some green algae (i.e., *Spirogyra* and *Draparnaldia*)), or *Cladophora* (~20%) in the presence or absence of perlid stoneflies (*Paragnetina media* and *Acroneuria abnormis*). Each of the six treatment combinations was replicated six times, resulting in 36 separate experimental units (channels).

Water in the 36 vinyl gutters (each 2.8 m long, 0.1 m wide, 7 cm high) used as channels was drawn from the East Branch of the Maple River with a Monarch pump. Stream water was first diverted to five head tanks (eight channels per head tank) before reaching channels. Netting (<0.1-mm mesh) over inlets to the head tanks prevented the introduction of unwanted macrobenthic animals into experimental channels. Both the head tanks and valves at the head of each channel allowed us to finely regulate discharge in the flow-through channels. Schematics of channel setup used to assay drift were similar to those illustrated in Holomuzki (1996). Water in each channel first flowed through a 0.8-m-long substrate-free section and then through a 0.5-m-long section containing substrate and caddisflies. These sections were separated by a dam of clay tiles (5 × 5 cm) stacked on end to help collect incoming FPOM in the substrate-free section and thus prevent downstream nets from clogging. A drift net placed 1.0 m downstream from the substrate-containing section (and 0.5 m from channel end) captured drifting caddisflies. The drift net created a ~0.7-m-long standing wave, so the 1-m-long gap between substrates and net ensured that any dam effects created by the net did not disrupt flow patterns in substrate-containing sections. Drift nets consisted of knee-high nylons (K-mart brand) supported by an inserted piece of PVC pipe (8–10 cm long, 6 cm in diameter). The mouth of the net was supported and expanded by hardware cloth (1.27-cm mesh) and glued with silicon sealant to the bottom and sides of the channels, conforming to the rectangular shape of the channels.

On 30 July 1996, 2 days after nets were glued in place, we added seven to nine flat cobbles (6–9 cm in diameter) covered with a thin algal biofilm, a thick periphyton mat, or *Cladophora* to channels (n = 12 for each algal treatment). Biofilm-covered and *Cladophora*-covered cobbles were collected from the Maple River and picked free of macroinvertebrates. Cobbles for the periphyton mat treatment had been collected from the Maple River 25 days earlier and placed in artificial streams (not used in the experiment) in conditions (i.e., grazer free, currents of ~35 cm·s^{-1}, 10% incident light) that promoted algal accrual. Rocks in experimental channels were arranged linearly and spaced a few millimetres apart. After rock placements, caddisflies and stoneflies collected from the Maple River on the same day were added to channels at ~15:30. Caddisflies (~70% *Ceratopsyche sparna*; fifth instars) were first sorted into groups of 10 in plastic cups, and then, each channel was stocked with 10 individuals from one container. Stonefly larvae were sorted into groups of two and randomly assigned to channels. This ratio (5:1) of caddisflies to stoneflies can be found in benthic patches in the Maple River. Valves at channel heads were momentarily turned off upon additions. Caddisflies and then stoneflies (1–3 min later) were gently poured from cups over substrate-containing sections. Discharge (0.4 L·s^{-1}), current velocity (~35 cm·s^{-1}, 2–10 mm over rocks, as measured with a pitot tube), and temperature (16°C) were similar among all channels. At 08:30, ~17 h after animal additions, caddisflies and stoneflies were collected from both drift nets and cobble-containing sections and counted. Substrates were thoroughly hand sorted to retrieve all hydropsychids to help quantify mortality. Mortality was assessed by summing counts of recovered, dead caddisflies and those that were unrecovered. We assumed that unrecovered caddisflies were consumed by stoneflies. A two-way MANOVA was used to assess the effects of algal cover and stonefly presence on the interdependent response variables percent drift and mortality (arcsine transformed). Significant MANOVAs were followed by univariate

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ANOVAs and LSD multiple comparisons to determine the source of observed differences.

Experiment 3. Cladophera cover – stonefly interactions

The combined effects of varying amounts of *Cladophora* cover and stonefly presence on hydropsychid drift were assessed in larger-scaled artificial stream channels at the Stream Facility. By increasing channel size, we also increased patch size (i.e., number of rocks) and altered the spatial arrangement of rocks in channels relative to experiment 2.

Stream water used in the six channels (each 3.65 m long, 0.24 m wide, 0.24 m high), constructed of untreated pine sealed with marine varnish, was drawn from the East Branch of the Maple River with a Monarch pump. In this experiment, head tanks were not available for use; only valves at the head of each channel regulated discharge in the flow-through channels. As before, water in each channel flowed first through a substrate-free section and then through a substrate-containing section. A 1.3-m-long section of channel from valve heads to a small dam of wood (1.0 cm high, 1.0 cm wide) collected incoming FPOM. A 1.0-m-long section starting 0.5 m downstream of the dam contained *Cladophora*-covered cobbles (18–24) spaced ~1 cm apart and arranged in a rectangle. A brick (19 × 9 cm, 5 cm high) at the end of the channel and 0.5 m from the downstream end of the substrate-containing section adjusted water depths to 5 cm throughout each channel. Drift nets (mouth 47 × 33 cm, 363-μm mesh) at the downstream ends of channels captured all animals leaving substrate-containing areas.

The combined effects of *Cladophora* cover and stoneflies on hydropsychid drift were examined on seven different dates (26–29 June and 9, 11, and 30 July1997) using the same procedures on each date. Rocks (surface area (mean ± 1 SE) 73 ± 8 cm^2, n = 20) with varying amounts of *Cladophora* cover were collected from the Maple River, hand-picked free of invertebrates, and placed in substrate-containing sections of channels. Two channels contained rocks with a low amount *Cladophora* cover (4–13%), two with a medium amount of cover (20–65%), and two with a high amount of cover (80–95%). Average *Cladophora* amounts over each substrate patch per channel were estimated visually by two or more individuals, averaged, and recorded. Caddisfly larvae from the Maple River were randomly sorted into groups of 25 in plastic cups and added to the upper one half of substrate-containing sections at 15:00. About 3–5 min after caddisfly additions (~80% *Ceratopsyche slossonae*, fifth instars), two perlid stoneflies randomly assigned to one of the two channels containing each *Cladophora* cover amount were added to the center of the substrate patch. Discharge was reduced momentarily by ~90% to prevent rapid displacement by the current and to facilitate settlement of both caddisflies and stoneflies on substrates. After 24 h, caddisflies and stoneflies captured in nets and remaining in substrates were counted in each channel. Caddisfly mortality was assessed as in experiment 2. Each of the six treatments was replicated on each of the seven dates using different caddisflies, stoneflies, and rocks for each replicate. Assignment of *Cladophora* levels and stoneflies to channels was done randomly for each replicate. Discharge (2 $L \cdot s^{-1}$), current (19 $cm \cdot s^{-1}$ 2 cm from the channel bottom), and temperature (16.5–18 °C) were uniform between channels on each trial. A two-way MANOVA was used to assess the effects of *Cladophora* cover and stonefly presence on arcsine-transformed drift and mortality rates (SYSTAT version 7.0). Results were then interpreted employing univariate tests and LSD multiple comparisons. Because *Cladophora* abundance varied slightly within each of the three cover treatments, we also used regression analysis to evaluate the relationship between percent drift and *Cladophora* abundance on rocks.

Experiment 4. Benthic settlement

We conducted a field experiment to assess how rock size and *Cladophora* cover jointly affect benthic colonization by hydropsychid larvae. Forty circular aluminum trays (15.25 cm in diameter, 3 cm deep) filled with either biofilm-covered gravels, biofilm-covered cobbles, *Cladophora*-covered gravels, or *Cladophora*-covered cobbles (n = 10 for each treatment) were used as colonization patches. Trays were filled so that rock contents were even with the tops of the trays. Rocks ~2 cm in diameter were placed in gravel-containing treatments, whereas six or seven rocks 6–9 cm in diameter were added to cobble-containing treatments. *Cladophora* covered ~15% of the upper surface area of rocks in *Cladophora* treatments. Because *Cladophora* only grows on the tops of rocks, we placed a ~1-cm-deep layer of *Cladophora*-free gravel under *Cladophora*-covered gravels. Gravel was not added to cobble-containing treatments. All trays were placed in a single riffle in the Main Branch on 25 July 1996 and countersunk so that tray contents were flush with the streambed. By placing all trays in the same riffle, and hence roughly in the same current velocity (~45 $cm \cdot s^{-1}$ 2 cm from the bottom), we could assess the effects of substratum size and algal cover, without the effects of current, on hydropsychid colonization. Trays were arranged in a rectangular pattern but staggered so that no two trays were immediately behind each other in consecutive rows. Each of the 10 rows contained one replicate of each substrate–algal treatment, and treatments were randomly placed within rows. Trays were 0.7 m apart within rows, and rows were spaced at 1.0-m intervals.

Trays were removed after 7 days and all contents preserved in 70% ethanol. Caddisflies were removed from rocks by wet sieving and by hand sorting. Numbers of hydropsychids colonizing the different substrate–algal cover treatments were compared using two-way ANOVA. Counts of stoneflies were also recorded in each tray to assess the relationship between substrate use by caddisflies and predator presence.

Results

Dispersal factors

Experiment 1. Drift from instream enclosures

Hydropsychid drift rates varied widely (1.7–58.4%) among enclosed substrates (Table 2). Larval drift was significantly affected by both stonefly density and substrate type (gravel) (Table 3). Specifically, drift rates were negatively related to stonefly density and positively related to percent gravel. Drift rates were not related to percent *Cladophora* cover. Hydropsychid densities approached ~1000 individuals·m^{-2} at two sites but were typically ≤150–160 individuals·m^{-2} (Table 1).

Experiment 2. Epilithic algae–stonefly interactions

Both amount and type of algae on rocks and stonefly presence affected drift of hydropsychid larvae in artificial streams (Table 4A). Drift rates were uniform across substrates covered with a thin biofilm, a thick periphyton mat, or *Cladophora* (~20%) when stoneflies were absent (Fig. 2A). However, when stoneflies were present, drift was higher from biofilm-covered cobbles than from the other cover treatments ($F_{1,30}$ = 3.83, P = 0.060). Mortality rates followed a similar pattern. Hydropsychid mortality was low (1.7–5.0%) in all three algal treatments when stoneflies were absent (Fig. 2B). However, mortality differed significantly among treatments when stoneflies were present ($F_{1,30}$ = 56.16, P < 0.001), being highest in the biofilm and periphyton mat treatments (both ~50%) and lowest in the *Cladophora* treatment (LSD comparison).

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Table 2. Physical and biotic data from enclosed substrates at the six study sites in the East (E) and Main (M) branches of the Maple River.

Enclosure site and experiment	*Cladophora* (%)	Gravel (%)	Current velocity ($cm \cdot s^{-1}$)	Hydropsychid density ($individuals \cdot m^{-2}$)	Hydropsychid drift (%)	Stonefly density ($individuals \cdot m^{-2}$)
Robinson Rd. (E)						
1	10	33	12	153±9	1.7	19±3
2	10	90	11	53±9	48.6	25±6
3	15	33	24	59±28	4.3	31±6
Plains Rd. (E)						
1	3	30	14	9±3	29.9	6±0
2	3	75	9	100±43	22.7	6±6
3	3	30	10	84±16	3.1	19±6
Douglas Lake Rd. (E)						
1	0	50	15	188±19	55.3	0±0
2	0	30	20	134±34	3.8	13±0
3	3	30	25	862±343	0.8	19±0
Old Spillway (M)						
1	25	85	9	278±47	44.3	0±0
2	3	75	6	125±31	4.1	6±0
3	5	45	7	44±12	0.0	0±0
Woodland Rd. (M)						
1	25	40	19	159±66	15.5	0±0
2	3	65	9	16±9	58.4	0±0
3	40	10	23	934±40	13.1	0±0
Gregory Rd. (M)						
1	2	20	7	97±34	9.4	6±0
2	20	35	14	31±19	29.9	0±0
3	7	5	11	81±68	17.6	0±0

Note. Density data are means ± 1 SE.

Table 3. Multiple regression results relating hydropsychid drift to *Cladophora* cover, gravel abundance, and stonefly density in enclosed substrates ($R^2 = 0.402$).

Regression analysis				
Variable	Regression coefficient ± 1 SE		*t*	*P*
Cladophora	–0.085±0.319		–0.27	0.793
Gravel	0.457±0.200		2.29	0.038
Stonefly density	–11.845±5.515		–2.15	0.050
ANOVA				
Source of variation	SS	df	*F*	*P*
Regression	1630.4	3	3.14	0.059
Residual	2423.5	14		

Note: Percentages of *Cladophora* and gravel were arcsine transformed, whereas density was $\log(x + 1)$ transformed for analyses.

Experiment 3. Cladophora cover – stonefly interactions

Propensity to drift varied significantly among treatments with different amounts of *Cladophora* cover (Fig. 3A; Table 4B). Drift was highest from rocks with low amounts of *Cladophora* and similar between rocks with medium and high *Cladophora* cover (LSD comparison). Regression analysis similarly showed that drift rates decreased as amount of *Cladophora* cover on rock surfaces increased ($R^2 = 0.631$, $P < 0.001$) (Fig. 4). Stoneflies, however, had no detectable effects on hydropsychid drift ($F_{1,36} = 0.019$, $P > 0.75$) (Fig. 3A), although they did significantly affect caddisfly mortality ($F_{1,36} = 16.13$, $P < 0.001$) (Fig. 3B). When stoneflies were present, mortality was higher in the low *Cladophora* cover treatment than in the other two treatments. Intensity of stonefly predation on caddisflies was apparently mediated by extent of algal cover, as indicated by a significant *Cladophora* × stonefly interaction ($F_{2, 36} = 3.813$, $P = 0.031$).

Experiment 4. Benthic settlement

Colonization rates varied significantly among substrates (Table 5). Numbers colonizing *Cladophora*-covered rocks were significantly higher than those colonizing biofilm-covered rocks (Fig. 5). Rock size, at least those two used in this experiment, had no apparent effect on settlement rates. Most (>90%) larvae colonizing each of the four rock size – epilithic algae treatments were *Ceratopsyche sparna*. Stoneflies, found in seven of the 40 colonization trays, were evenly distributed among the four substrate treatments (ANOVA, $P = 0.20$).

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Table 4. MANOVA results for effects of (A) algal cover type (biofilm, periphyton mat, or *Cladophora*) and stonefly presence or absence and (B) *Cladophora* abundance (low, medium, or high) and stonefly presence or absence on drift and mortality of hydropsychid larvae in artificial stream experiments.

Source	df	Wilks' λ	*F*	*P*
A				
Algal cover	4,58	0.620	3.92	0.007
Stoneflies	2,29	0.348	27.15	<0.001
Algal cover × stoneflies	4,58	0.717	2.63	0.043
B				
Cladophora abundance	4,70	0.593	5.22	0.001
Stoneflies	2,35	0.691	7.84	0.002
Cladophora × stoneflies	4,70	0.786	2.24	0.073

Discussion

Predator effects on hydropsychid drift decisions appear to depend on algal habitat conditions. The use of drift as a predator avoidance mechanism was favored in structurally simple habitats. Results from both artificial stream experiments indicate that stoneflies can increase hydropsychid drift from substrates with comparatively less epilithic algal cover. However, retreat availability may interact with algal cover to determine stonefly-induced drift responses. Only partially constructed retreats were present in channels of both artificial stream experiments; apparently <1 day, the duration of our experiments, was not enough time to complete retreat construction. In contrast, numerous fully constructed retreats were present on substrates in our field enclosures, where stonefly density and hydropsychid drift were inversely related. These results suggest that once retreats are fully constructed, larvae respond to stonefly presence by remaining in retreats rather than by drifting. This interpretation is supported by Fuller and Rand's (1990) observation that captures by *Agnetina* were significantly lower when larvae (*Cheumatopsyche* sp.) were in retreats than when free-living. Thus, stoneflies may be most influential in provoking drift when larvae first resettle to the benthic environment and (or) when retreat construction is incomplete.

Epilithon density may strongly influence whether larvae remain in a substrate patch after resettlement, particularly when stoneflies are present. Larval additions to our artificial streams simulated benthic resettlement after drifting. Drift rates in artificial streams with stoneflies were significantly higher from rocks with a thin biofilm than from those with a thick periphyton mat or *Cladophora*. Apparently a biomass-rich periphyton mat on rocks can enhance surface microtopography enough to provide sufficient microhabitat refugia from predatory stoneflies and hence decrease larval drift. Yet, *Cladophora* cover had no effect on hydropsychid drift in our field enclosure experiment. These apparently contradictory results may again be partially explained by the interacting effects of predation risk and retreat presence. Larvae may be particularly vulnerable to predators when first resettling to the benthic environment. Until a retreat is constructed, the dense thick filaments of *Cladophora* may provide larvae protection from both vertebrate and invertebrate predators (see Holomuzki and Short 1988; Power 1990). Further, a dense epilithon of micro- and (or) macroalgae may offer hydropsychid larvae other potential benefits. For example, green algae filaments may be an important construction material for retreats, as suggested by Mackay and Waters (1986) and as evidenced in both our field enclosures and artificial streams. Further, *Cladophora* serves as a substrate for epiphytic diatoms (e.g., *Cocconeis*) (e.g., Dodds and Gudder 1992), which may be used as a food source by hydropyschids. Thus, the concomitant effects of predator deterrence, availability of retreat materials, and en-

Fig. 2. Mean (+1 SE) percent (A) drift and (B) mortality of hydropsychid larvae on cobbles covered with either a thin biofilm, a thick periphyton mat, or *Cladophora* (~20% surface cover) with or without stoneflies (n = 6 for each of the six algal–predator treatment combinations).

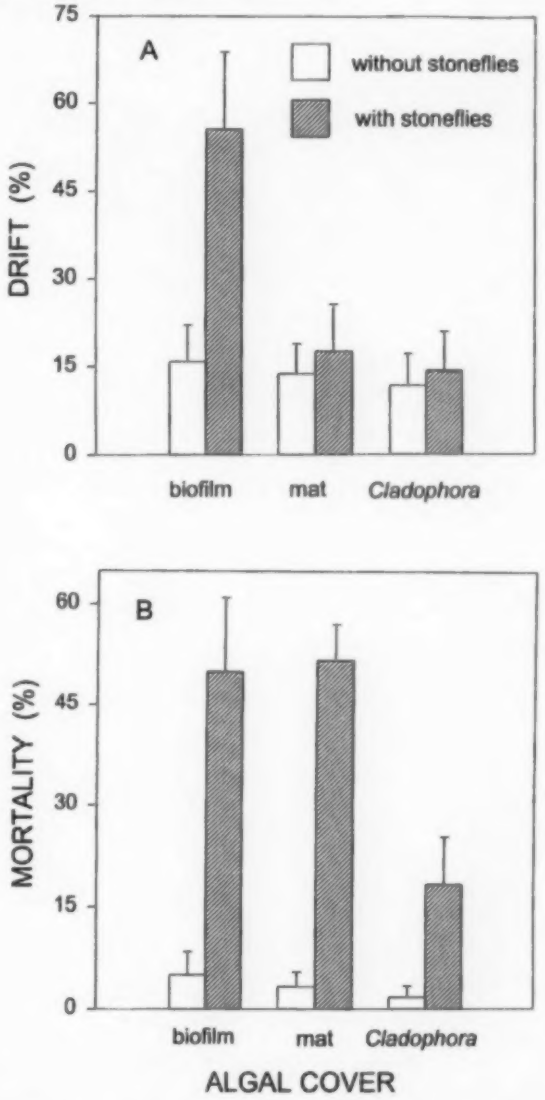

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Fig. 3. Mean (+1 SE) percent (A) drift and (B) mortality of hydropsychid larvae on cobbles covered with either low (4–13%), medium (20–65%), or high (80–95%) amounts of *Cladophora* with or without stoneflies (n = 6 for each *Cladophora*–predator treatment).

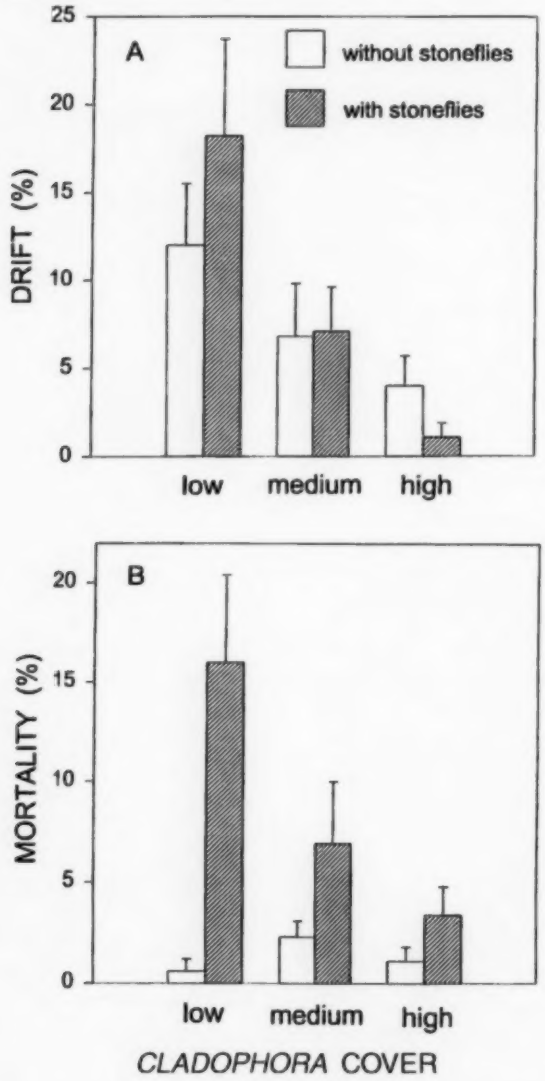

Fig. 4. Hydropsychid drift as a function of percent *Cladophora* cover on cobble surfaces.

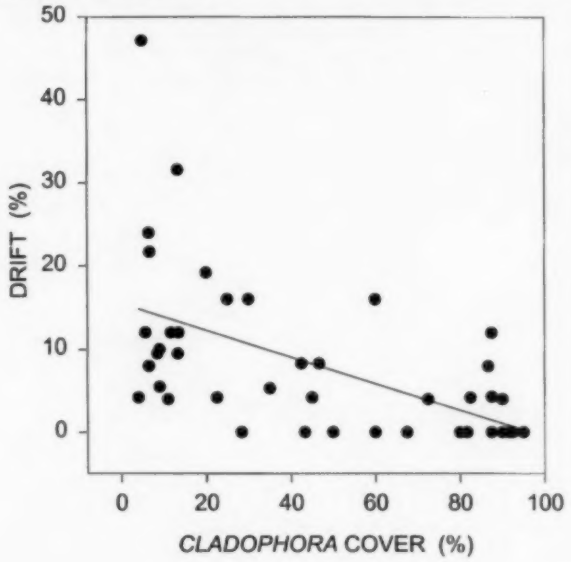

Table 5. Two-way ANOVA comparing colonization of hydropsychid larvae on different rock sizes (gravel or cobble) with different types of epilithic algae (biofilm (diatom dominated) or *Cladophora*).

Source of variation	SS	df	F	P
Rock size	3.025	1	0.004	0.948
Epilithic algae	17514.225	1	24.753	<0.001
Rock size × algae	483.025	1	0.683	0.414
Error	2547.500	36		

hanced foraging profitability may explain the reduced drift rates from rocks with a biomass-rich epilithon.

The relative amount of *Cladophora* on rock substrata also affects hydropsychid dispersal decisions. Average amount of *Cladophora* cover on substrates in our field enclosures was ~10% (limits 0–40%). *Cladophora* patches with much higher percent cover occur in the Maple River. However, these patches are associated with large substrates (boulders) and fast flows, both of which precluded construction of enclosures. We were able to broadly vary percent *Cladophora* (~5–90%) on rock substrata in our artificial streams (experiment 3). By taking this approach, we found that hydropsychid drift was significantly inversely related to percent *Cladophora* cover. Moreover, the in situ colonization experiment (experiment 4) demonstrated that the presence of relatively small amounts of *Cladophora* (~15% cover) can greatly increase settlement rates on rock substrata. These results imply that hydropsychid drift responses and settlement patterns are linked to *Cladophora* growth phases and thus are temporally variable. *Cladophora* is a mid- to late-successional species (Dodds and Gudder 1992) that typically forms thick mats on large rocks in summer (Dudley et al. 1986; Dodds 1991). It follows that other factors, such as flow disturbance (flooding) and herbivory, that can decrease *Cladophora* biomass and (or) retard algal community succession (Power 1990; Dudley and D'Antonio 1991) will also indirectly affect hydropsychid drift and colonization. Thus, the influence of *Cladophora* on hydropsychid dispersal may have both spatial and temporal components.

Results from our field enclosures indicate that substrate size also affects hydropsychid drift. Drift rates were relatively high when enclosed substrates were gravel dominated. High drift rates may be indicative of an unpreferred habitat. Benke et al. (1991) found that hydropsychid drift rates increased when suitable habitat (snags) was less available.

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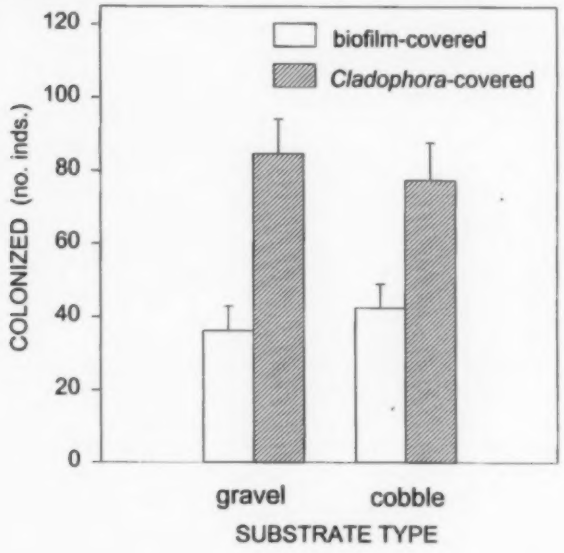
Fig. 5. Mean (+1 SE) number of hydropsychid larvae per tray colonizing biofilm-covered gravels, biofilm-covered cobbles, *Cladophora*-covered (~15%) gravels, and *Cladophora*-covered cobbles (n = 10 for each algal–substrate size treatment).



Gravels may be less preferred than larger stones because of their instability in higher flow (e.g., Grimm and Fisher 1989). Further, for filter-feeding caddisflies, gravels may produce a different, less favorable microhydraulic environment for retreat construction and food gathering than larger stones (Wetmore et al. 1990). However, gravels may provide better protection from some predators than larger-sized stones. Michael and Culver (1987) found that *Hydropsyche bronta* preferred gravels (13–33 mm in diameter) over larger substrata (53–175 mm in diameter) when predaceous stoneflies (*Acroneuria lycorias*) were present. When stoneflies were absent, no preference between the two stone sizes was observed. They also reported that substratum size alone did not affect predator-induced drift.

An understanding has emerged that many factors affect dispersal decisions (e.g., Mackay 1992; Rader 1997; and many others). In order to understand and predict why some factors influence dispersal while others do not, it is important to determine how, why, and when these factors interact. Many of the interacting mechanisms that underlie dispersal behaviors also likely drive population and community dynamics. Accordingly, multifactor approaches to understanding the causes of individual dispersal should also help us understand processes and predict responses at higher organizational scales.

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The reappearance of spawning Atlantic herring (*Clupea harengus*) on Aberdeen Bank (North Sea) in 1983 and its relationship to environmental conditions

Ad Corten

Abstract: Evidence is presented in support of the hypothesis that the reappearance of spawning Atlantic herring (*Clupea harengus*) on Aberdeen Bank in 1983, after an absence of 16 years, was related to an increased Atlantic inflow in the area. Two Atlantic copepod species, *Metridia lucens* and *Candacia armata*, showed a simultaneous increase in the years when spawning herring returned to Aberdeen Bank. During the late 1960s, both species declined at the time when the spawning population on Aberdeen Bank disappeared. Earlier work has demonstrated that an increased Atlantic inflow results in a southward displacement of plankton concentrations and feeding herring in the northwestern North Sea. I hypothesize that such a southern distribution of the herring stock, caused indirectly by the increased Atlantic inflow, influenced recruit spawners to choose the nearby Aberdeen Bank as their spawning ground in 1983. Fluctuations of the spawning populations in the northern North Sea during earlier decades are explained by switches of recruitment between the northern and southern population, as a result of variations in latitudinal distribution of the recruiting year-class.

Résumé : Des observations sont présentées à l'appui de l'hypothèse selon laquelle la réapparition de harengs géniteurs sur le banc Aberdeen en 1983, après une absence de 16 ans, était liée à une augmentation du flux atlantique dans cette zone. Deux espèces de copépodes de l'Atlantique, *Metridia lucens* et *Candacia armata*, ont vu leur population augmenter simultanément au cours des années où des harengs sont revenus frayer sur le banc Aberdeen. À la fin des années 1960, ces deux espèces ont décliné au moment de la disparition de la population de géniteurs sur le banc Aberdeen. Des travaux antérieurs ont montré qu'une augmentation du flux d'eau de l'Atlantique donnait lieu à un déplacement vers le sud des concentrations de plancton et des harengs qui s'en nourrissaient dans le nord-ouest de la mer du Nord. Je formule l'hypothèse qu'un tel prolongement vers le sud de la répartition du stock de harengs, découlant indirectement de l'augmentation de l'afflux d'eau de l'Atlantique, avait porté les géniteurs à choisir le banc Aberdeen voisin comme frayère en 1983. Les fluctuations des populations de géniteurs dans la partie nord de la mer du Nord au cours des décennies précédentes sont expliquées par des alternances du recrutement entre la population nord et la population sud qui résultaient de variations en latitude de l'aire de répartition de la classe d'âge en recrutement.

[Traduit par la Rédaction]

Introduction

During the annual Atlantic herring (*Clupea harengus*) larvae survey in the North Sea in 1983, large numbers of herring larvae were detected on Aberdeen Bank, an area where no spawning had been observed during the previous 16 years but where heavy spawning used to take place in the 1950s and 1960s. In these earlier years, the "Buchan" fishery for prespawning and spawning herring in the vicinity of Aberdeen Bank was one of the most important herring fisheries in the North Sea. The reappearance of herring larvae on Aberdeen Bank in 1983 has been described in detail by several authors (Kiørboe and Johanson 1986; Munk et al. 1986; Richardson et al. 1986). None of these, however, addressed the question of the origin of the spawning herring on Aberdeen Bank or what made them suddenly choose Aberdeen Bank as a spawning ground. The reappearance of larvae on Aberdeen Bank happened at a time when the North Sea herring stock was increasing after a period of stock depletion, and most scientists probably considered the spawning at Aberdeen Bank as a kind of "overflow" from the population at Shetland/Orkney, the spawning grounds of which are situated about 300 km further north (Fig. 1).

Observations on the Buchan population in earlier decades, however, indicated that this stock did not depend merely on an overflow of individuals from the Shetland/Orkney population. Although the two populations were very similar in their distribution and annual migrations (Fig. 2), their stock sizes had shown long-term variations that were independent, or even the opposite, of each other. Glover (1955, 1957) showed that the driftnet fishery for prespawning herring off the Scottish east coast shifted south between 1947 and 1954, causing prosperity to the southern (Buchan) ports of Peterhead and Fraserburgh, while the fishery at Shetland col-

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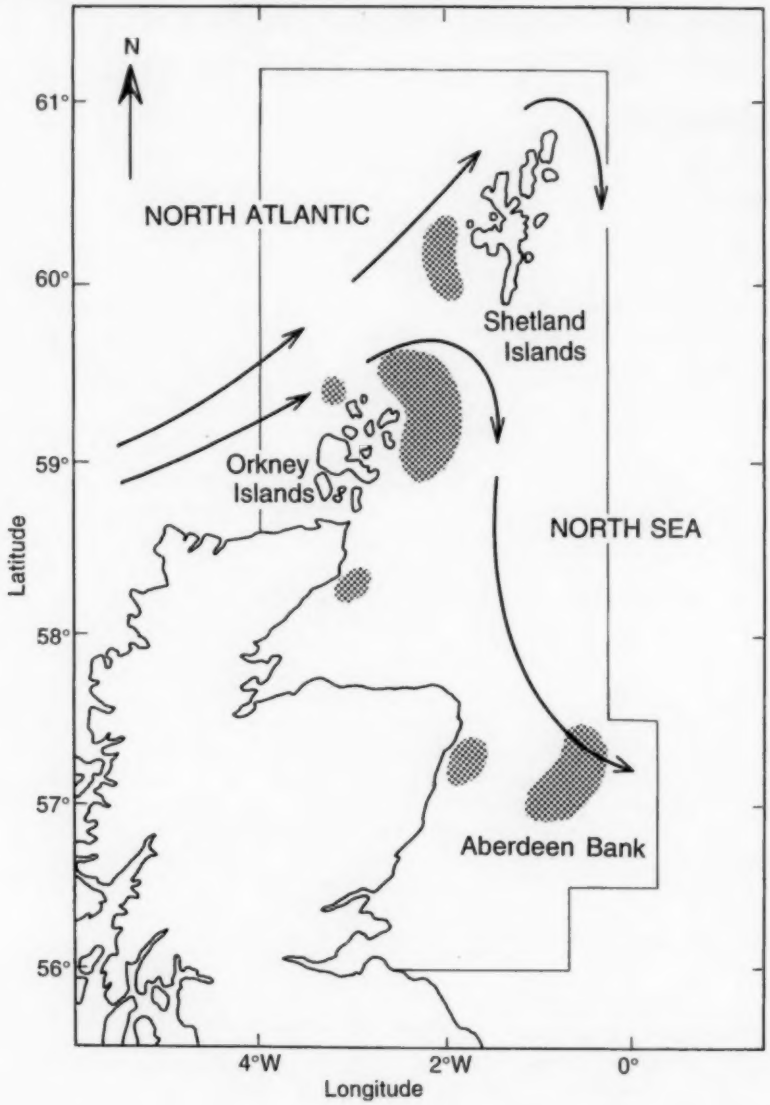
Fig. 1. Herring spawning areas in the northwestern North Sea (adapted from Rankine 1986). Also indicated are the main inflows of Atlantic water. The sampling area for herring larvae is indicated by the box.



lapsed. The increased herring catches on the Buchan grounds coincided with an increased quantity of herring food in this area and an earlier appearance of plankton species associated with oceanic water. After the boom of the 1950s, the Buchan fishery near Aberdeen Bank declined in the 1960s, while the fishery at Shetland recovered. Bainbridge and Forsyth (1972) and Bainbridge et al. (1978) showed that these developments coincided with a decline of the main food organisms of the herring, *Calanus finmarchicus* and *Spiratella* (= *Limacina*) *retroversa*, in the Buchan area after 1958. The virtual disappearance of the Buchan prespawning fishery from 1966 to 1969 coincided with an extreme scarcity of these food items in the Buchan area. The annual fluctuations in the abundance of *Calanus* and *Spiratella* in the Buchan area appeared to be largely determined by variations in the strength of Atlantic inflow into the northwestern North Sea.

The decline of the fishery in the Buchan area was accompanied by a reduction of the larval production in this area. Saville (1971, 1978) presented the results of Scottish herring larvae surveys from 1951 to 1967, which showed that the abundance of larvae on Aberdeen Bank was high in the early 1950s but gradually decreased to zero in 1967. At the same time, the abundance of larvae in the region of the Orkneys strongly increased.

The results of these investigations show that the Buchan

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population in earlier years changed independently from the Shetland/Orkney population. Spawning on Aberdeen Bank was at a maximum when the Atlantic inflow into the northwestern North Sea seemed to be strong and when food abundance was high in the Buchan area in the months prior to spawning. This suggests that the intensity of spawning at Aberdeen Bank is a function, either directly or indirectly, of the Atlantic inflow into the northwestern North Sea.

If the spawning of herring at Aberdeen Bank is indeed dependent on the Atlantic inflow, the reappearance of larvae in this area in 1983 must have been a symptom of increased Atlantic inflow into the northwestern North Sea. We shall investigate this hypothesis by looking at data on hydrography and the occurrence of plankton indicator species. Furthermore, the identity of herring populations in the northern North Sea is considered in the light of the reappearance of larvae on the Aberdeen Bank. Was the reappearance due to the revival of a rudimentary population that had always persisted in this area, or was it due to colonization by herring that came from elsewhere?

The question whether individual spawning populations represent self-contained stocks is important from a management point of view. It was discussed intensively in the North Sea area in the 1950s and 1960s (Postuma and Zijlstra 1958; Zijlstra 1958, 1964, 1969, 1973; Parrish and Sharman 1959; Kreft 1963; Schumacher 1963), but the interest in the subject declined in later years. In the meantime, North American scientists have developed new concepts about stock structure in herring (Iles and Sinclair 1982; Smith and Morse 1990; Stephenson and Kornfield 1990; McQuinn 1997). The reappearance of a spawning population at Aberdeen Bank provides an opportunity to apply these new concepts also to the North Sea situation.

Material and methods

Spawning grounds

The intensity of spawning on the various grounds in the northern North Sea was estimated from the abundance of small herring larvae, taken from ICES surveys conducted from 1972 to 1993 (Anonymous 1985). A database with the complete results of all surveys from 1972 to 1993 is kept at the Marine Laboratory in Aberdeen. The international sampling effort strongly declined in the early 1990s, and 1992 was the last year with a full coverage of the northwestern North Sea. The distribution and abundance of herring larvae are only an indirect index of spawning intensity on the various spawning grounds. The survival of herring spawn and larvae is not constant from year to year, and also, the distance that the larvae have drifted away from their hatching position may differ between years. To reduce the error introduced by differential mortality and drift, only the category of larvae <10 mm has been considered. Applying an average daily growth rate of 0.15 mm and a size at hatching of 6.7 mm, these larvae will be between 1 and 22 days old. Assuming a residual drift of 3.4 km/day, the larvae may have drifted up to 75 km away from their hatching ground (Munk et al. 1986). The majority of the larvae, however, will be <22 days old, and their position at the time of capture will be <75 km away from their hatching ground.

Data on numbers of larvae per square metre were extracted from the database for all sampling stations in the northwestern North Sea between 56°00′N and 62°00′N and between 4°00′W and 01°00′E (Fig. 1). This area, which includes the main spawning grounds at Orkney/Shetland and Aberdeen Bank, was covered each year between 1972 and 1992. For each year, mean densities per station were calculated for the first and second half of September, and then, the values for both half-monthly periods were averaged. This procedure gives equal weight to both halves of the month, irrespective of sampling effort. Finally, the yearly values were averaged over 5-year periods.

Age distributions of spawning herring

Age distributions of spawning herring taken on Aberdeen Bank were available from Dutch commercial catches in this area. During the first years when herring resumed spawning on Aberdeen Bank, the Dutch fleet apparently did not notice the concentrations of spawning herring, and no catches were taken. It was only from 1986 onwards that a fishery for spawning herring developed on Aberdeen Bank and that samples of spawning herring from this region were collected under the routine sampling scheme for commercial catches of the Dutch fisheries research institute RIVO-DLO. Unfortunately, no samples of spawning herring were obtained from the Shetland/Orkney spawning grounds. In this area, the spawning grounds are situated mainly within the 12-mile fishing limit, and there was no Dutch fishery on spawning herring. The Marine Laboratory in Aberdeen could not provide age data for Shetland/Orkney spawners in the 1980s either.

Hydrographic data

Temperature and salinity data for the northwestern North Sea were kindly provided by the ICES hydrographic service. Data were extracted for the month of August, which is the start of the spawning season in the northern North Sea. Sampling intensity was low during the period 1972–1992, and insufficient data were available to construct a time series for the Aberdeen Bank area. As a substitute, a series of surface temperature and salinity data was constructed for the entire northwestern North Sea, extending from 02°00′W to 02°00′E and from 56°00′N to 61°00′N. Even for this larger area, the number of samples in August dropped below 50 in most of the years after 1980, and for the years 1989–1991, no samples were available at all.

Plankton indicator species

The oceanic influence in the northwestern North Sea can also be estimated from the occurrence of plankton indicator species. A large amount of plankton data for the northwestern North Sea have been collected over the years from ships of opportunity by the Continuous Plankton Recorder (CPR) survey, presently operated by the Sir Alister Hardy Foundation for Ocean Science (SAHFOS) in Plymouth. Data from all surveys since 1948 are accessible from a computerized database. A full description of the methods used in the CPR programme is given in Warner and Hays (1994).

Based on the results of earlier studies (Rae and Rees 1947; Lee and Ramster 1977; Fransz et al. 1991; Krause et al. 1995), two copepod species, *Metridia lucens* and *Candacia armata*, were selected as indicators of oceanic influence in the northwestern North Sea. These species enter the North Sea each year in late summer with the inflowing Atlantic water, and they disappear from the North Sea in the first half of the year.

Mean numbers per CPR sample (3 m^3) for *M. lucens* and *C. armata* were calculated for the northwestern North Sea for each month in the period 1948–1996. The northwestern North Sea was defined as the area between 56°N and 60°N and between 2°E and 4°W. The choice of a relative wide sampling area was necessary in order to obtain a sufficient number of samples in each month (15–20 on average). Annual values for both species were derived by taking the mean of the monthly indices. A combined index for *M. lucens* and *C. armata* was constructed by standardizing the annual values for both species (subtracting the mean and dividing by

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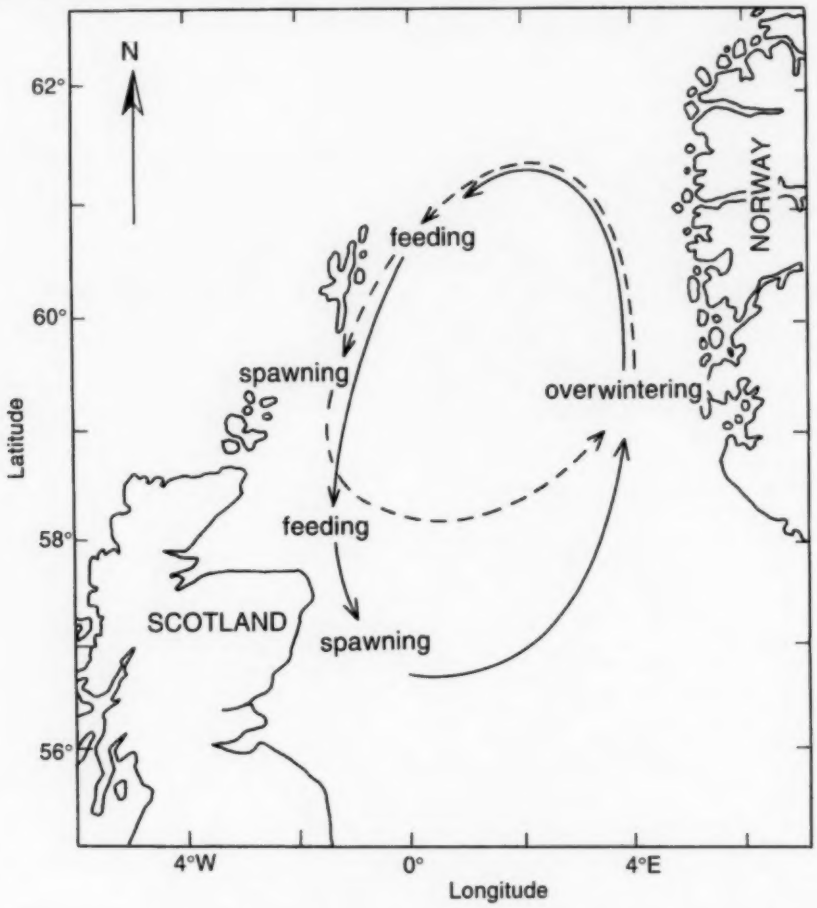
Fig. 2. Seasonal migrations of the Buchan (solid lines) and Shetland/Orkney (broken lines) herring populations.



the standard deviation) and taking the average of the two standardized values in each year.

Results

Larval abundance

After a period of complete absence from 1972 to 1982, herring larvae appeared again on Aberdeen Bank in 1983 and remained present in subsequent years (Figs. 3 and 4). The recolonization of the Aberdeen Bank spawning ground happened very abruptly. In the preceding year (1982), there had been some larvae at the inshore spawning area near Peterhead, but herring larvae had been found here during most of the earlier years, and their occurrence had never been followed by spawning at Aberdeen Bank. However, in 1983 and particularly in 1984, large numbers of larvae suddenly appeared on Aberdeen Bank, making this area as important as the more northern spawning grounds at Shetland/Orkney. The Aberdeen Bank spawning ground retained a prominent position at least until the last year of full survey coverage (1992).

The charts of larvae distributions show that the spawning on Aberdeen Bank in 1983 was not the result of a gradual buildup of a small resident population in the area. In the years 1972–1980, not a single larva was taken on Aberdeen Bank during any of the surveys. The adult herring that spawned on Aberdeen Bank in 1983, therefore, could not have been born at this locality.

To investigate whether the spawning at Aberdeen Bank in 1983 was due to an overflow of the existing spawning population at Shetland/Orkney, the temporal trend of total larval abundance in the Shetland/Orkney area is compared with larval abundance at Aberdeen Bank (Fig. 5). It is seen that the appearance of larvae on Aberdeen Bank in 1983 did not coincide with an increased abundance at Shetland/Orkney; in fact, larval abundance in the latter area had declined between 1978 and 1982. Consequently, the appearance of spawners on Aberdeen Bank in 1983 probably was not due to overcrowding of the existing spawning grounds at Shetland/Orkney. The later increase of larval abundance at Shetland/Orkney in 1988–1990 indicated that these grounds could in fact accommodate far more spawners than they did in 1983.

Age compositions of spawning herring

Age compositions of spawning herring on Aberdeen Bank

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Fig. 3. Mean distribution of herring larvae by periods of 5 years (except for the first and last periods). The legend represents densities per square metre.

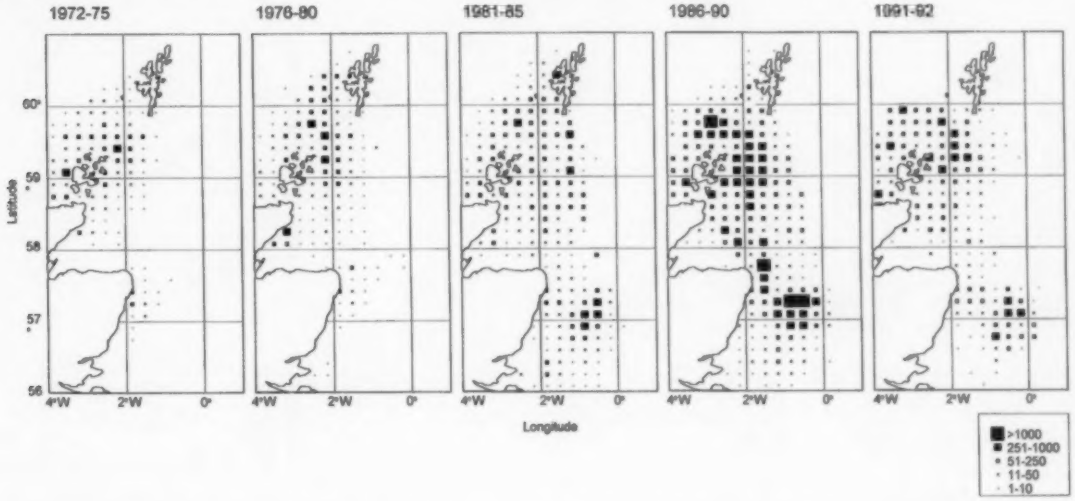
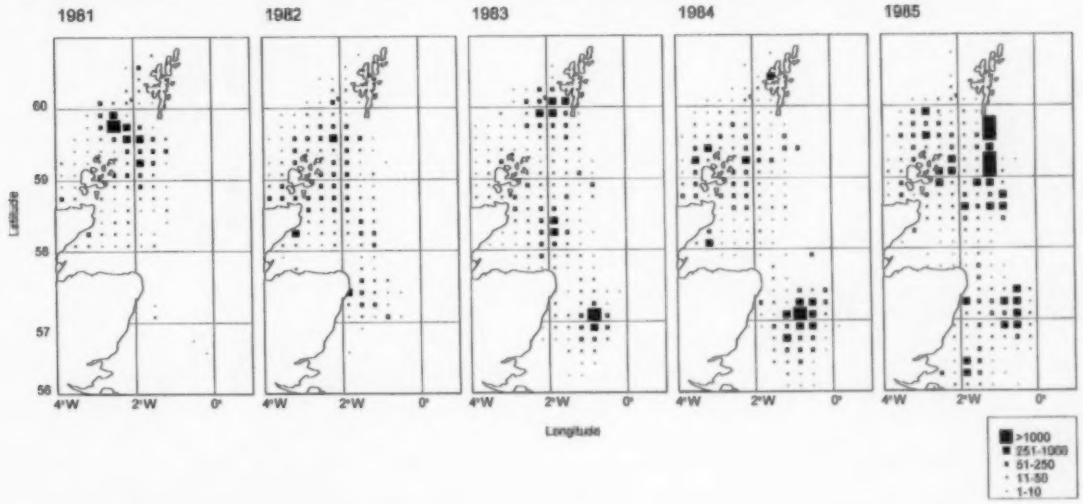


Fig. 4. Annual distribution of herring larvae in the period 1981–1985. The legend represents densities per square metre.



were available from 1986 to 1993 (Fig. 6). These age compositions show that the spawners in 1986–1988 were mostly young fish aged 3–5 years, with a small component of fish aged 6–7 years Assuming that older herring return to their place of first spawning, the age compositions for 1986 and subsequent years provide indirect estimates of the age of the spawners in 1983–1985. The oldest herring sampled in 1986–1988 belonged to year-classes 1979 and 1980. This suggests that the spawning on Aberdeen Bank in 1983 was started by these two year-classes (aged 3 and 4 years at the time).

In the years 1989–1992, the proportion of 3-year-olds among the spawners sharply declined. The abundance of larvae at Aberdeen Bank in this period was not markedly different from the abundance during the preceding 6 years. This indicates that there was no major change in population size compared with the preceding period. The reduced proportion of 3-year-olds among the spawners therefore indicates a reduction of the number of herring recruiting at this age. All the year-classes recruiting during this period appeared in greater numbers from age 4 onwards. This means that the age of first maturity in the population was changing from age 3 to age 4 or older. Maturity distributions for the total North Sea stock show a delayed recruitment for the years 1992 and 1993, but not particularly for the years 1989–1991. Apparently, the changes in age at first recruitment at Aberdeen Bank were not representative of those in the entire North Sea stock.

During the period 1988–1992, a series of strong year-classes (1985–1987) recruited to the overall North Sea stock (Fig. 7). Considering the large increase in larval abundance at Shetland/Orkney in 1988–1990, it is reasonable to assume

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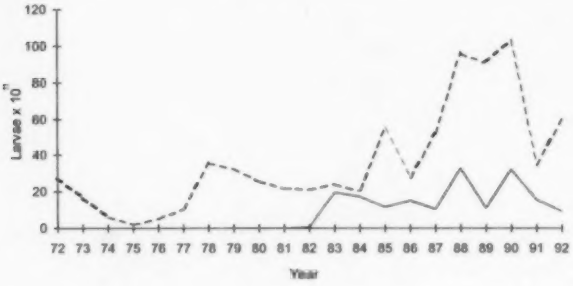
Fig. 5. Total abundance of herring larvae at Aberdeen Bank (solid line) and Shetland/Orkney (broken line). Aberdeen Bank has been defined as between 56°30′N and 57°30′N and between 00°20′E and 01°00′W and Shetland/Orkney as the area north of 58°N.



that more herring of these strong year-classes recruited to Shetland/Orkney than to Aberdeen Bank.

Hydrographic data

Mean values of surface temperature and salinity for August in the northwestern North Sea are presented in Fig. 8. The data do not indicate a significant change in hydrographic conditions around 1983 that might explain the renewed attractiveness of Aberdeen Bank as a spawning ground for herring.

Plankton indicator species

Time series for the two Atlantic indicator species in the northwestern North Sea, *M. lucens* and *C. armata*, were constructed for the entire period (1948–1996) for which data were available (Fig. 9). During this period, *M. lucens* reached high values in the years 1955–1964 and also in 1982–1992. The period 1965–1981 was characterized by a marked scarcity of this species. The series for *C. armata* shows a similar pattern. Also in this species, two periods of relatively high abundance are separated by a period of low abundance during the second half of the 1960s and all of the 1970s.

In addition to the parallel trends in annual abundance, the species exhibit similar changes in seasonal occurrence in the North Sea. In the years 1965–1981, the appearance of both species in the North Sea was delayed by 1 or 2 months during this period (Fig. 10). Considering the fact that these species are recognized as indicators for Atlantic inflow in the northwestern North Sea, their parallel changes most likely signify multiannual variations in the influx of Atlantic water into this area.

To compare the abundance of herring larvae on Aberdeen Bank with the occurrence of Atlantic plankton in the northwestern North Sea, the time series of larval abundance indices from the ICES surveys (1972–1992) was extended with Saville's (1971) data for the period 1951–1967. It is doubtful whether Saville's (1971) data are directly comparable with the later series from the ICES surveys in view of possible differences in survey coverage, sampling methods, and sampler efficiency. However, Saville's (1971) data at least provide a qualitative index of the abundance of larvae on Aberdeen Bank.

Fig. 6. Percentage age compositions of spawning herring on Aberdeen Bank in 1986–1993.

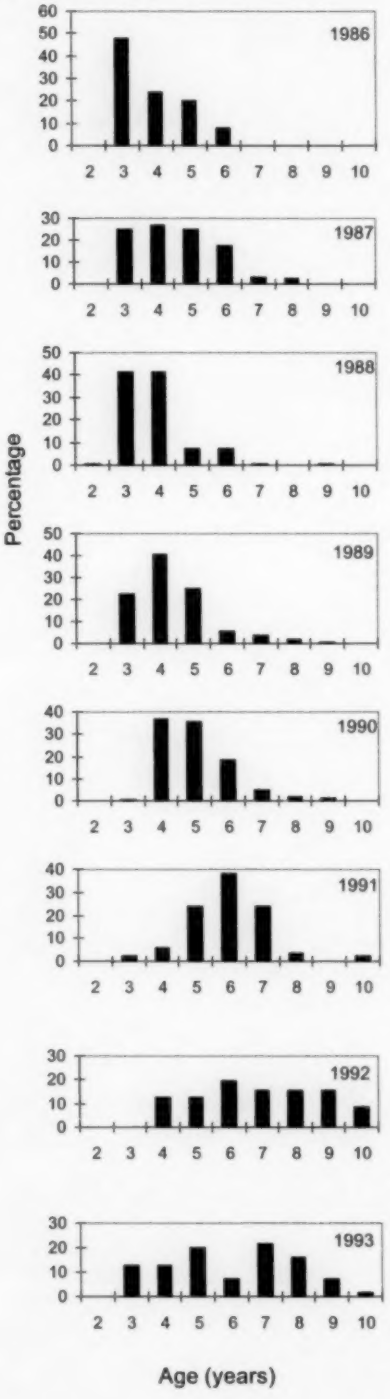


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Fig. 7. Recruitment to the total North Sea stock at age zero (data from Anonymous 1998).

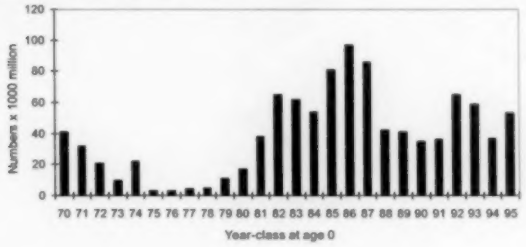
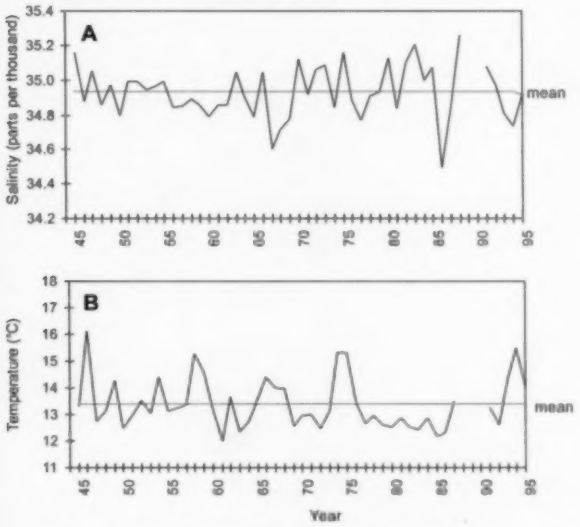


Fig. 8. Surface (A) salinity and (B) temperature in the northwestern North Sea during August.



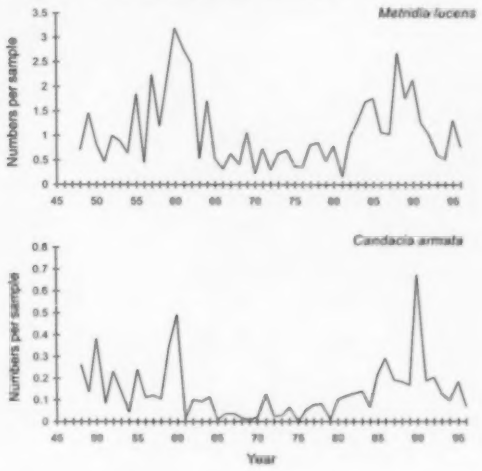
The comparison of the data series for herring larvae with the combined index for *Metridia* and *Candacia* (Fig. 11) shows that the period in which herring larvae were absent from Aberdeen Bank (1965–1982) coincided with the period of reduced abundance of the two Atlantic indicator species. Spawning at Aberdeen Bank was resumed in 1983; the first year in which the *Metridia*/*Candacia* index reached above-average values after 18 years.

Discussion

Environmental connection

Data on Atlantic plankton species presented in this paper support the hypothesis that the revival of the Aberdeen Bank spawning population in 1983 was related to an increased Atlantic inflow in the northwestern North Sea. Other authors have described an earlier period of expansion of the Buchan population at a time of increased Atlantic inflow (Glover 1955, 1957) and a decline of the population at a time when the Atlantic inflow was reduced (Bainbridge and Forsyth 1972). We have evidence, therefore, that revival of the spawning population on Aberdeen Bank in the 1980s was another episode in a long-term cycle of expansion and contraction of the Buchan population in response to variations in Atlantic inflow into the area.

Fig. 9. Mean annual abundance per CPR sample of *M. lucens* and *C. armata* in the northwestern North Sea.



The lack of hydrographic evidence for the postulated changes in Atlantic inflow is probably due to the limited amount of data available. Earlier authors (Bainbridge and Forsyth 1972) found that variations of Atlantic inflow could be detected from salinity data only by looking at smaller areas within the northwestern North Sea. Hydrographic sampling in the post-1980 period was too limited to allow the calculation of mean salinities for these smaller areas.

The relationship between of the Atlantic inflow and spawning at Aberdeen Bank could be either direct or indirect. A strong Atlantic inflow might affect temperature and salinity on Aberdeen Bank, thereby improving hydrographic conditions for spawning. However, it is hard to envisage how a change in hydrographic conditions on Aberdeen Bank would induce new herring spawn in this area. It is unlikely that prespawning herring each year make a quick survey of all available spawning grounds to determine where hydrographic conditions are optimal for spawning.

More likely, a strong Atlantic inflow will affect the choice of spawning grounds indirectly by changing the geographical distribution of prespawning herring. Bainbridge et al. (1978) reported that the abundance of planktonic food in the Buchan area was partly dependent on a strong Atlantic inflow in summer. The same applied to the latitudinal distribution of the herring, with a strong inflow resulting in a southern distribution of the herring. Apparently, a strong inflow of Atlantic water results in a southward displacement of the area of maximum plankton production, and hence in a similar displacement of the feeding herring. In years with a strong inflow, the herring will often find themselves in a relatively southern position at the end of the feeding season. As the spawning season in the northern North Sea follows immediately after the feeding season, recruit spawners will be inclined to choose a spawning ground that is not too far from the position where they stop feeding. In the case of a southern distribution, the Aberdeen Bank is the most likely choice.

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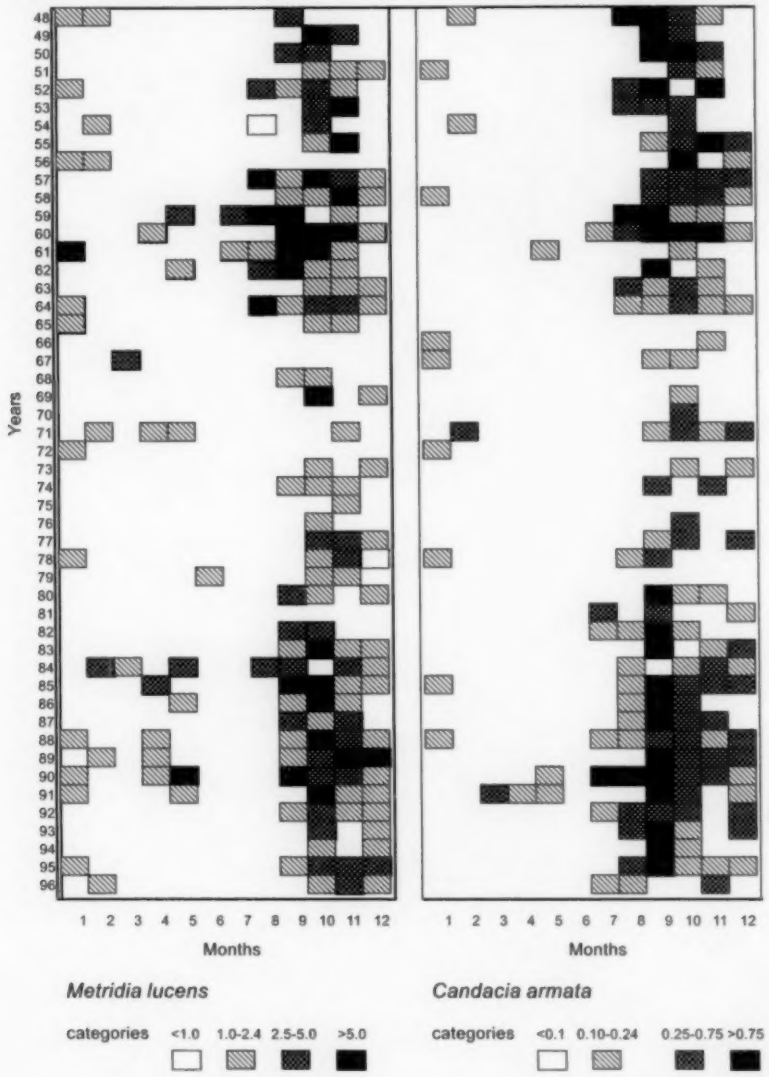
Fig. 10. Abundance of *M. lucens* and *C. armata* in each month during the years 1948–1996. Categories are numbers per CPR sample.



The strength of the Atlantic inflow, however, is not the only parameter that determines the latitudinal distribution of herring. In the summers of 1988–1990, herring in the North Sea had a very northerly distribution (Corten 1999), despite a strong Atlantic inflow as indicated by the *Metridia/Candacia* index. The northerly distribution of the herring coincided with high water temperatures and scarcity of *C. finmarchicus*, the main food item of the herring. During these years, the distribution of *C. finmarchicus* was confined to the northern part of its normal range (Fromentin and Planque 1996; Planque and Fromentin 1996). The high temperatures and associated changes in plankton distribution appear to have had an overriding effect on the distribution of the herring in these years.

The northerly distribution of the entire herring stock during the summers of 1988–1990 may explain the relatively low recruitment of new spawners to Aberdeen Bank, despite the high Atlantic inflow. If the herring were distributed far north at the end of the feeding season, recruit spawners would have been inclined to chose Shetland/Orkney instead of Aberdeen Bank as their place of first spawning. Data on larval abundance suggest that the Shetland/Orkney area indeed received the bulk of the recruit spawners in these years.

Population identity

The reappearance of a spawning population on Aberdeen Bank, 16 years after the last herring larva had been caught in this area, has implications for our understanding of the stock structure of North Sea herring. In the North Sea area, scientists have long discussed the question of whether the individual spawning populations were self-contained stocks or whether they drew their recruits also from neighbouring

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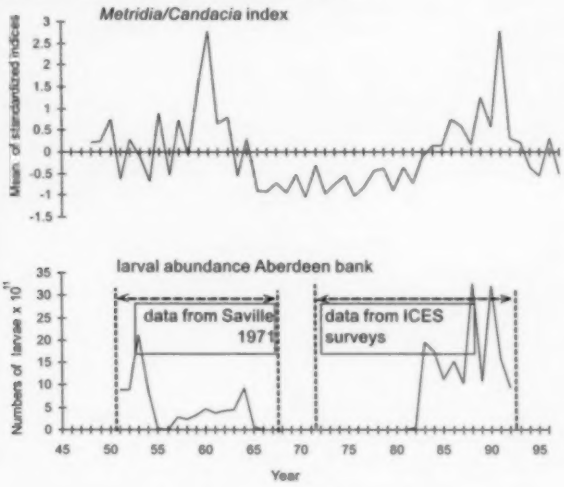
Fig. 11. Abundance of herring larvae on Aberdeen Bank compared with a combined index for *Metridia/Candacia*, representing the strength of Atlantic inflow.



populations. The hypothesis of self-contained stocks was advocated by Postuma and Zijlstra (1958) and Zijlstra (1958, 1964, 1969, 1973), whereas Parrish and Sharman (1959), Kreft (1963), and Schumacher (1963) produced arguments for an exchange of recruits between populations. The discussion about the genetic identity of spawning populations was not restricted to the North Sea area.

In North America, the main exponents of the theory of self-contained stocks were Iles and Sinclair (1982), whereas Smith and Jamieson (1986) refuted the evidence for genetic isolation of spawning populations. McQuinn (1997) gave an extensive review of the literature on the subject, both in Europe and in North America. The discussion in North America was stimulated by the reappearance of herring larvae on Georges Bank in 1986, 9 years after these formerly important spawning grounds had been deserted. Stephenson and Kornfield (1990) argued that this was a case of resurgence, i.e., a revival of a rudimentary population that had never completely disappeared, whereas Smith and Morse (1990) suggested that the new spawners had no direct connection to the spawning population that formerly existed in this area.

Turning to our observations on Aberdeen Bank spawners, we come to the same conclusion as Smith and Morse (1990) did for the Georges Bank herring. The herring that spawned on Aberdeen Bank in 1983 cannot have been descendants from a rudimentary population that always survived in this area. Extensive ICES larvae surveys in 1972–1981 found no evidence of any larvae production on Aberdeen Bank. Therefore, the herring that spawned in this area in 1983 must have been born elsewhere. The implication is that the Aberdeen Bank population is not (and has never been) a self-contained stock. Apparently, juvenile herring born at other localities in the North Sea can spawn at Aberdeen Bank and thereby become a member of the local population. If this applies to the Aberdeen Bank population, it is probably also true for other spawning populations in the North Sea.

The apparent lack of "homing" in (at least some of) the recruit spawners raises the question of how the identity of existing spawning populations is maintained. Some of these populations have been known for decades or even centuries, and there must be some mechanism by which new generations continue to recruit to these traditional spawning places. As early as 1962, Wynne-Edwards had drawn attention to the role of tradition in maintaining population identity in birds, mammals, and fish. He identified the separate populations of North Sea herring as one case in which the transmission of migration patterns by learning could play an important role in maintaining population identity (and success). Many years later, Corten (1993) arrived at the same conclusion with regard to the maintenance of these populations.

Recently, McQuinn (1997) applied the principle of social transmission of migration patterns in his "adopted migrant" hypothesis. This theory postulates that individual spawning populations within a larger area all belong to one "metapopulation" and that the individual populations draw their recruits from a common pool of juveniles. Juvenile herring recruit to one of the populations by schooling with older, experienced herring and by following these to one of the traditional spawning sites. When spawning in this locality for the first time, the recruit spawners become attached to the ground and return there in subsequent years. Spawning populations are thus perpetuated by the transfer of tradition from one generation to the next and not by instinctive homing of first-time spawners.

Since older herring have already developed an attachment to one of the existing spawning site, they will not be the ones that colonize new spawning areas. If spawners are found in a new area, these fish will normally belong to the recruiting year-class (McQuinn 1997). Our observations on the age composition of Aberdeen Bank spawners confirm this theory, as it appears that the first spawning in 1983 was conducted by recruit spawners. The same observation was made on Georges Bank, where the first spawners in 1986 belonged to the recruiting 1983 year-class (Stephenson and Kornfield 1990; McQuinn 1997).

The adopted migrant theory predicts that colonization of new spawning areas will occur when established populations become unstable following a population collapse or recruitment boom (McQuinn 1997). The case of the Aberdeen Bank spawners illustrates how the process might actually work. A relatively minor change in hydrography (only detectable from plankton indicator species) probably resulted in a southward displacement of feeding herring. The recruiting year-class, which is always distributed at the southern fringe of the population, lost contact with the older stock component that stayed behind in the Shetland/Orkney area ("knowing" that it had to spawn there within a few weeks). Without the guidance of older herring, the recruit spawners turned to the nearest place where bottom substrate and hydrographic conditions met their instinctive requirements for a spawning ground, and a new spawning population was established. The scenario illustrates that no large instability of the population was required, nor a major change in hydrography. All that was needed was the separation of a number of recruit spawners from the older stock component during the prespawning period. This is most likely to occur when the recruiting year-class is large in relation to the existing adult stock, i.e., a recruitment boom.

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The colonization of new spawning grounds illustrates that recruit spawners are free, within the limits of the metapopulation, to choose their own spawning ground. However, in most years, this freedom will not be expressed in the establishment of new spawning sites, but merely in switches between existing spawning areas. Normally, the recruiting year-class will be mixed with herring 1–2 years older that have already developed an attachment to one of the existing spawning areas. The choice of spawning area by recruit spawners will depend on the older herring that they associate with during the prespawning phase. If, in a particular year, recruit herring in the North Sea have a northerly distribution, they are likely to mix with experienced spawners from Shetland/Orkney and consequently follow these fish to the Shetland/Orkney spawning grounds. Conversely, a southerly distribution of the recruiting year-class will mix them with members of the Aberdeen Bank population, and hence lead them automatically to the Aberdeen Bank spawning grounds. Shifts in recruitment between Shetland/Orkney and Aberdeen Bank, based on this mechanism, must have been responsible for the contrasting developments in both populations in earlier decades.

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Isotopic composition of otoliths as a chemical tracer in population identification of sockeye salmon (*Oncorhynchus nerka*)

Y.W. Gao and R.J. Beamish

Abstract: The feasibility of stable oxygen and carbon isotope ratio ($\delta^{18}O$ and $\delta^{13}C$) analyses in sagittal otoliths of sockeye salmon (*Oncorhynchus nerka*) was tested by analyzing the seasonal and annual otolith zones of 44 samples collected from different localities in the northeast Pacific coast. The $\delta^{18}O$ and $\delta^{13}C$ values of these otoliths ranged from −14.23 to +1.62‰ and from −15.18 to −3.17‰, respectively. The $\delta^{18}O$ variations can be divided into two stages from freshwater (−14.2 to −2.5‰) to marine (−2.5 to +1.6‰) that were consistent with the life history of sockeye salmon from juvenile to adult stages. The transition occurred after age 1, during which the timing of seaward migration of smolts was different. The marine component of the isotope variation in sockeye salmon otoliths (ages > 2) was uniform but showed a consistent and strong shift towards oceanic changes around 1996. Thus, $\delta^{18}O$ and $\delta^{13}C$ values of otoliths can be potentially used as a chemical tracer in population identification, and their marine portions can be used in the study of ocean environmental changes.

Résumé : La possibilité d'effectuer des analyses des rapports des isotopes stables de l'oxygène et du carbone ($\delta^{18}O$ et $\delta^{13}C$) dans les otolithes sagittaux du saumon rouge (*Oncorhynchus nerka*) a été vérifiée par l'étude des zones saisonnières et annuelles des otolithes de 44 échantillons prélevés en différents endroits de la côte nord-est du Pacifique. Les $\delta^{18}O$ et $\delta^{13}C$ de ces otolithes variaient respectivement de −14,23 à +1,62 ‰ et de −15,18 à −3,17 ‰. Les variations du $\delta^{18}O$ peuvent être divisées en deux étapes, de l'eau douce (−14,2 à −2,5 ‰) à l'eau de mer (−2,5 à +1,6 ‰), qui correspondent au cycle vital du saumon rouge, de l'état de juvénile à celui d'adulte. La transition se produisait après l'âge 1, période pendant laquelle le moment de l'avalaison des saumoneaux était différent. La composante marine de la variation isotopique des otolithes des saumons rouges (âge > 2) était uniforme, mais présentait un décalage constant et important en fonction des changements océaniques vers 1996. Par conséquent, les $\delta^{18}O$ et $\delta^{13}C$ pourraient servir de traceurs chimiques pour l'identification des populations et leur composantes marines pourraient servir à l'étude des changements de l'environnement océanique.

[Traduit par la Rédaction]

Introduction

Otoliths are laminated calcium carbonate structures located in the inner ears of teleost fish and have long been used in aging (Beamish and McFarlane 1987). The theory and practice of using otoliths in stable isotope analysis are rooted in Urey's (1947) hypothesis and the experiments that calcium carbonates are precipitated in oxygen isotopic equilibrium with the surrounding waters in which the organism (including fish) lived and thus retained the isotopic records of the life history of the animal (e.g., Epstein et al. 1953; Friedman and O'Neil 1977; Grossman and Ku 1986). Yearly, seasonal, and even daily increments in otoliths (Pannella 1971) are produced throughout the life of a fish and are geochemically inert after precipitation. Thus, stable oxygen isotope ratios ($^{18}O/^{16}O$) extracted from otoliths can provide unique information about habitat alteration, water temperature, migration, and decadal-scale ecosystem changes experienced by the individual fish (e.g., Devereux 1967; Mulcahy et al. 1979; Nelson et al. 1989; Kalish 1991. In contrast, stable carbon isotope ratios ($^{13}C/^{12}C$) in otoliths are deposited in isotopic disequilibrium with the ambient seawater and can record changes in maturation of the fish and the dietary shifts (Schwarcz et al. 1998).

Sockeye salmon (*Oncorhynchus nerka*) are commercially the most valuable of the five species of Pacific salmon in North America (Forrester 1987). The recent decline in abundance of this species has raised concerns in both the scientific community and the public. In this paper, we report the results of stable isotope ratio analysis from the proxy of otoliths. Previous stable isotope studies on sockeye salmon have concentrated on the trophic hierarchy of salmonids and used flesh as proxy materials in general (Welch and Parsons 1993; Perry et al. 1996; Kline et al. 1998). Compared with otoliths, the flesh of a fish is convenient to sample, but it is impossible to get a proper time series for retrospective studies. Kalish (1991) reported oxygen and carbon isotopic composition in otoliths of Australian salmon (*Arripis trutta*) from captive and wild fish, but his data also lack the connection with a time series. As the life of sockeye salmon is so dependent, from year to year, on food sources and oceanic

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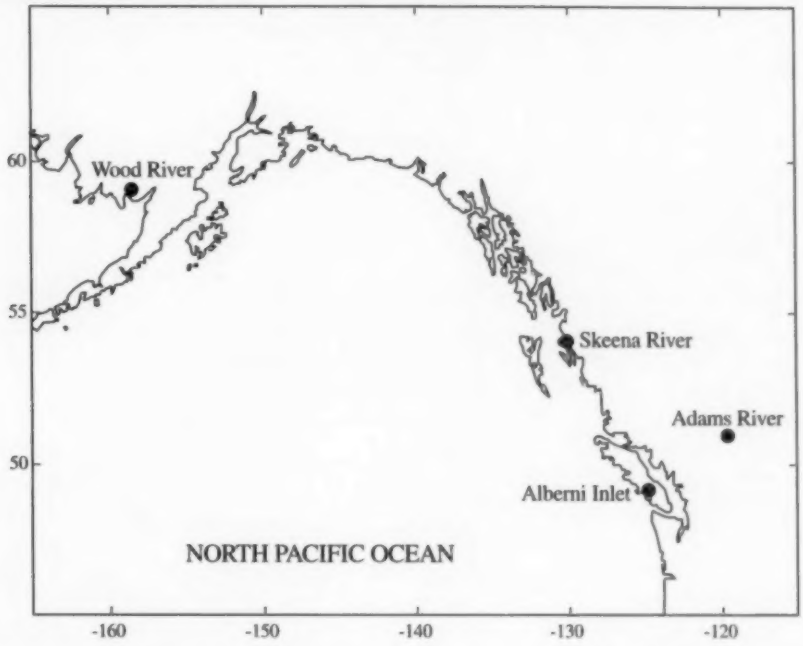
Fig. 1. Location map showing the sampling sites in the northeast Pacific.



conditions, it is necessary to analyze the isotope data within a suitable time series, whether at seasonal or annual scales. In particular, there have been no attempts to analyze stable isotopes in archived otoliths that could provide information on the growth conditions and survival of sockeye salmon in the past. Fortunately, the recent development of microsampling (at annual and intraannual/seasonal scales) coupled with mass spectrometric techniques capable of analysis of microgram quantities of carbonate has resulted in a powerful new tool in otolith geochemistry (Patterson et al. 1993; Gao 1999). The first purpose of the study was to examine the lifetime isotopic composition of individual otoliths to evaluate whether the characteristics of isotope variations are consistent with the life history and behavior of sockeye salmon. The second goal was to examine the use of isotope data as chemical tracers in population identification of sockeye salmon and the potential of using the marine portion of otoliths for ocean environmental and ecological studies.

Materials and methods

Sockeye salmon otoliths (15–20 from each locality) were collected throughout the northeast Pacific coast. These sagittal otolith pairs were taken in the summer of 1998 from July 10 to August 1 from the Wood River in Bristol Bay of Alaska, Skeena River, British Columbia, and Alberni Inlet on Vancouver Island (Fig. 1). Sockeye salmon scales and background information, such as fork length, weight, and sex, were also taken during the sample collection. In addition, 57 sockeye salmon otoliths from the Adams River, British Columbia, were gathered in the fall of 1996. Although only 5–10 Adams River sockeye salmon were reported here, the detailed isotopic analyses from translucent and opaque otolith zones (winter and summer) provided an example for the feasibility and future application of the technique.

Otolith samples of sockeye salmon were first cleaned in a supersonic water bath for about 15 min, rinsed with ethanol, and then dried naturally. The samples were first placed in an aluminum mold and then embedded in pigmented resin. The embedding medium that we used consists of a common fiberglass resin, black pigment, and a TR mold release. After hardening, the block was removed from the mold and sectioned into rectangles for polishing. Each otolith section was polished using an ECOMET 3 variable-speed grinder–polisher with self-adhesive paper (240–2400 grit). At the final stage, an AP-Cloth was used for surface polishing with 0.3 μm Alumina powders. Care was taken to polish only until annual growth zones became visible. Using the Dremel microsampling method (Gao 1999), cores of about 50 μg of aragonite were removed from the otolith surface. These samples were generally obtained from the annual otolith zones (annuli), except for the first 1 or 2 years in which single translucent and opaque (winter and summer) otolith zones were taken. To avoid any confusion with the age designation, sockeye salmon ages are reported as years old or total ages throughout this paper (Burgner 1991). The aragonite powder samples were analyzed on a VG Optima mass spectrometer at the School of Geography and Geology, McMaster University, using an Autocarb carbonate preparation device. All the measurements are reported in the standard δ notation (per mill), $\delta^{18}O = \{[(^{18}O/^{16}O)_X/(^{18}O/^{16}O)_S] - 1\} \times 1000$, where X is sample and S is standard (Vienna Peedee belemnite). The precision of analyses is better than ±0.06‰ for both $\delta^{18}O$ and $\delta^{13}C$.

Results

The $\delta^{18}O$ and $\delta^{13}C$ values of sockeye salmon otoliths ranged from –14.23 to +1.62‰ and from –15.18 to –3.17‰, respectively. There were slight differences in the isotope values between different geographic areas (Table 1). Based on the early life study and aging, all the samples analyzed were divided into two major groups: freshwater and marine. The range in isotope values and the mean of each group are

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Table 1. Summary of stable isotope analysis of sockeye salmon otoliths in this study.

			$\delta^{13}C$ (‰)			$\delta^{18}O$ (‰)		
Settings	Localities	*N*	Range	Mean	SD	Range	Mean	SD
Freshwater (ages 0–1)	Wood River	10	−15.18 to −7.11	−12.76	2.22	−11.33 to −2.78	−8.36	2.30
	Skeena River	10	−14.41 to −7.91	−11.21	1.91	−13.54 to −3.74	−9.64	2.63
	Alberni Inlet	10	−14.99 to −7.08	−12.49	1.96	−9.55 to −2.37	−6.88	1.85
	Adams River	10	−13.29 to −9.92	−11.68	1.05	−14.23 to −9.30	−12.05	1.60
Marine (ages 2–5)	Wood River	7	−5.82 to −3.33	−4.21	0.58	−0.88 to +1.58	+1.06	0.60
	Skeena River	10	−5.75 to −3.17	−4.54	0.72	−0.48 to +1.62	+0.83	0.69
	Alberni Inlet	10	−6.57 to −3.44	−4.74	0.64	−1.49 to +1.61	+0.66	0.68
	Adams River	7	−5.01 to −4.23	−4.55	0.22	−0.16 to +1.12	+0.55	0.36

Fig. 2. $\delta^{18}O$ variations of individual otolith samples from the Adams River. The life history of sockeye salmon can be isotopically divided into two stages from freshwater to marine, and the transition occurred after age 1.

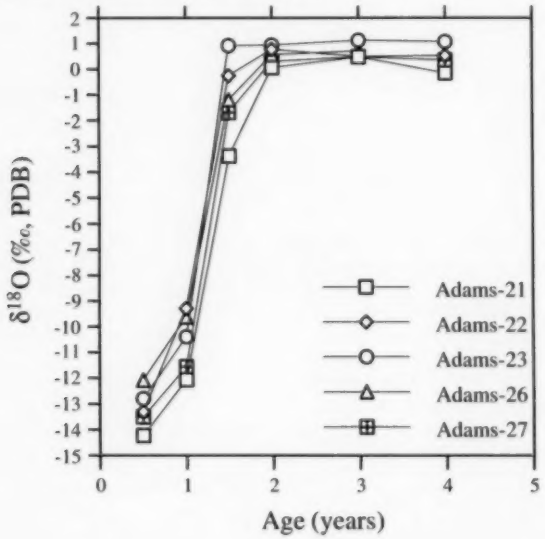
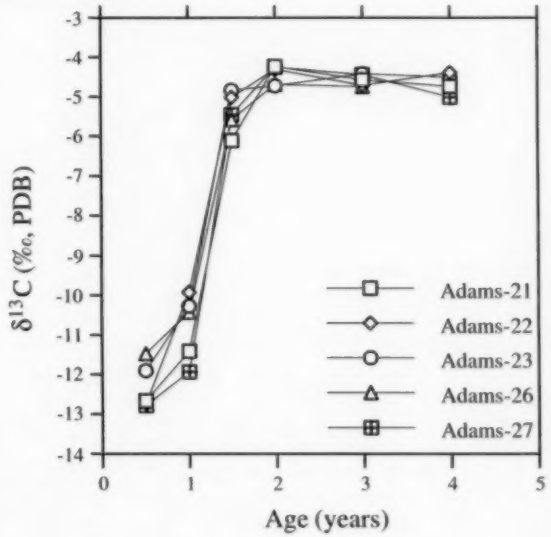


Fig. 3. $\delta^{13}C$ variations of individual otoliths from the Adams River showing a consistent trend in $\delta^{18}O$ in the lives of sockeye salmon.



slightly different (ANOVA, $F_{3,75} = 2.73$, $p = 0.050$). For example, the mean $\delta^{13}C$ value of Wood River sockeye salmon in freshwater was slightly lower than that of Skeena River sockeye salmon (−12.76 versus −11.21‰), whereas the mean $\delta^{18}O$ value of the marine group was higher (+1.06 versus +0.83‰). Overall, the variation in isotope values of sockeye salmon in freshwater environments was much larger than that in marine environments (freshwater SD from 1.60 to 2.63 and marine SD from 0.36 to 0.69) (Table 1).

The $\delta^{18}O$ values of individual Adams River sockeye salmon ranged from −14.23 to +1.12‰, very homogeneous from the first season to age 4 (Fig. 2). Because otolith growth is limited during the spawning run of adult sockeye salmon, we were unable to obtain discrete carbonate that represents that life history stage. However, based on $\delta^{18}O$ values, the life history of sockeye salmon can be divided into two stages: freshwater (−14.23 to −2.37‰) and marine (−1.49 to +1.62‰) (Table 1), the latter being relatively uniform. The transition started after age 1 in an estuarine environment (e.g., −2.5 to 0.5‰), during which the individual fish entering the sea were slightly different (between ages 1 and 2 in Fig. 2). The $\delta^{13}C$ variation of individual Adams River sockeye salmon was consistent with that of $\delta^{18}O$, ranging from −13.29 to −9.92‰ in freshwater and from −5.01 to −4.23‰ in marine settings (Fig. 3). A similar pattern and range of isotope variations (averaged from 5 to 10 fish) were shown for other localities (Fig. 4).

For the first season of sockeye salmon otoliths (winter in freshwater), the relationships of $\delta^{18}O$ and $\delta^{13}C$ in the neighboring areas, between Adams River and Alberni Inlet and between Skeena River and Wood River, are easily distinguished (Fig. 5). Because $\delta^{18}O$ variations are related to the physical properties of seawater, whereas $\delta^{13}C$ variations are related to trophic conditions, this separation appears to suggest that the isotopic signals can be used as a habitat index to distinguish the natal sources of different sockeye salmon populations.

Although $\delta^{13}C$ values of British Columbia coastal samples are slightly lower (Fig. 6), the marine portion of isotope variations in sockeye salmon otoliths showed a pronounced overlap among different populations from the Gulf of Alaska

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Fig. 4. Average isotope range and variation of sockeye salmon otoliths from different areas in the present study (sample size in parentheses). (*a*) Mean $\delta^{18}O$ values; (*b*) mean $\delta^{13}C$ values.

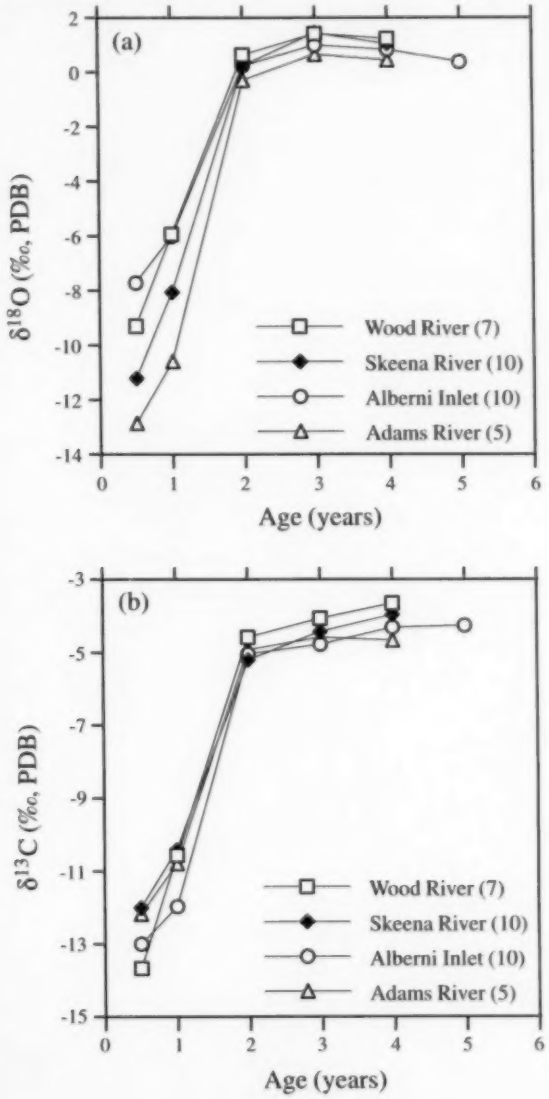
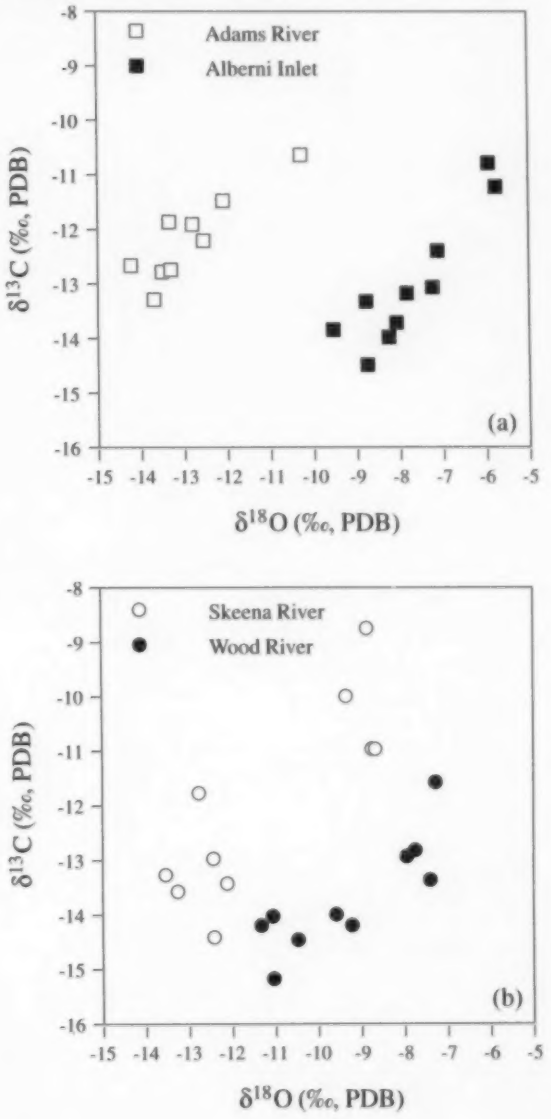


Fig. 5. Relationship between $\delta^{18}O$ and $\delta^{13}C$ of sockeye salmon otoliths from the first season. (*a*) Adams River and Alberni Inlet; (*b*) Skeena River and Wood River. See text for details.



to Vancouver Island (ANOVA, $F_{2,73} = 1.88$, $p = 0.160$). The overlap was probably due to similar timing of smolts moving into the sea around age 2, or mixing of sockeye salmon from different stocks during their oceanic pastures. The average isotope values (both $\delta^{13}C$ and $\delta^{18}O$) for different sockeye salmon stocks were consistent from ages 2 to 4, corresponding to the time series of calendar years 1995–1997 (Fig. 7). Over that period the mean $\delta^{13}C$ values of all sockeye salmon otoliths along the coast were steadily increasing, whereas the mean $\delta^{18}O$ values shifted around 1996. The shift of 0.6–0.8‰ in $\delta^{18}O$, if any, would represent a significant change in oceanic conditions of the North Pacific.

Discussion

As an anadromous fish, sockeye salmon spend most of their lives (2–5 years) in coastal and high-seas environments and, after maturity, return to their parent coastal streams to spawn (Foerster 1968; Burgner 1991). The isotopic composition of sockeye salmon otoliths did reveal anadromous stages from freshwater to marine settings in both $\delta^{18}O$ and $\delta^{13}C$ values (Figs. 2 and 3). It is known that $\delta^{18}O$ values in standard seawater are around 0‰ and in freshwater are generally lower than –10‰ (Craig 1961). Thus the $\delta^{18}O$ data from sockeye salmon otoliths are consistent with previous

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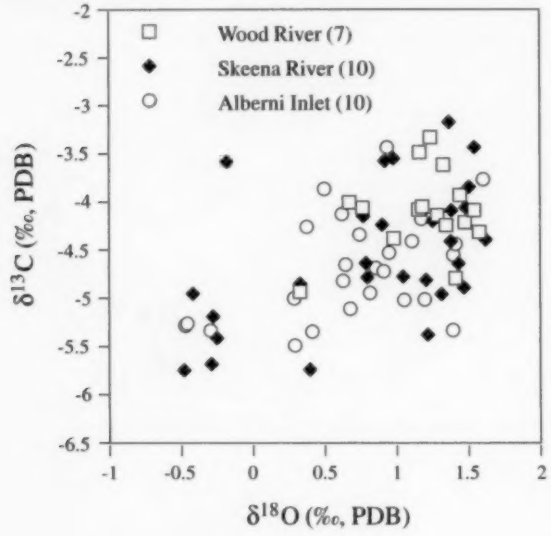
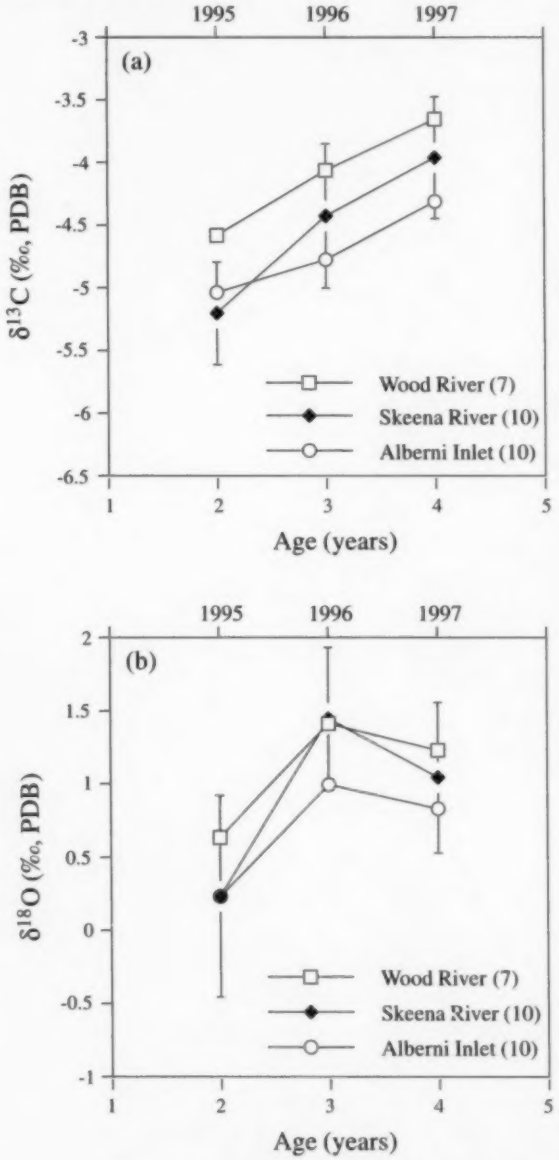
Fig. 6. Overlap of $\delta^{18}O$ and $\delta^{13}C$ from the marine portion of otoliths from different sockeye salmon populations.



studies of stable isotope geochemistry and fisheries biology. The $\delta^{13}C$ values are also consistent with the two stages of sockeye salmon life history, although their isotopic meanings in otoliths are not primarily related to water conditions (Schwarcz et al. 1998). The $\delta^{13}C$ values from –13.5 to –7.0‰ and from –7.0 to –3.5‰ (Fig. 4*b*) probably represent the isotope variations of planktonic crustaceans in the freshwater environment and pelagic plankton organisms in the marine environment (Welch and Parsons 1993).

The identification of sockeye salmon populations or stocks has been documented by tagging and some physical methods (Foerster 1968). From tagging, the locations of release and recapture of sockeye salmon in the ocean are recorded, and the subsequent movement of the fish can be followed. In addition to the cost, however, this "human-made" marker has the disadvantage of low recovery rates (<10‰ in general). The physical methods, such as scale patterns of sockeye salmon and morphological features of otoliths (Bugaev 1987), are dependent primarily on the examiner's experience and it is sometimes difficult to obtain quantitative measurements. Genetic markers, such as protein electrophoresis (Rutherford et al. 1992) and the new development of microsatellite DNA (Beacham and Wood 1999), have also been used in stock identification of sockeye salmon. Comments on the role of genetic markers and points for attention have been made by Ferguson and Danzmann (1998). Here, we suggest that stable isotope ratio analysis in otoliths could be used for population identification of sockeye salmon. The isotopic analysis generates a natural "chemical" marker, which can determine not only the origin of the fish (Fig. 5), but also the tracks of migration of individuals (Fig. 2). From the first seasonal isotope values between populations, we see that the Alberni Inlet sockeye salmon are more enriched in ^{18}O but depleted in ^{13}C than the Adams River sockeye salmon (Fig. 5*a*). In contrast, the Skeena River sockeye salmon salmon are more enriched in

Fig. 7. Average isotope variations of the marine portion of otoliths from different sockeye salmon populations (sample size in parentheses). (*a*) $\delta^{13}C$ variation: a stable increase from ages 2 to 4; (*b*) $\delta^{18}O$ variation: a shift around age 3 that corresponds to calendar year 1996.



^{13}C than the Wood River sockeye salmon and slightly depleted in ^{18}O (Fig. 5*b*). These isotopic differences may result from different $\delta^{18}O$ values in the natal rivers or lakes, or from the food sources of different residences or from both. If this is true, it will indicate that the freshwater discharge in the Adams River system has stronger influences than that on Vancouver Island (from $\delta^{18}O$) and that the trophic level of planktonic crustaceans is also slightly higher than the latter (from $\delta^{13}C$). Similar conclusions can be made for Skeena

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River and Wood River sockeye salmon. We did not know from the present study whether the isotopic technique would be effective in discriminating among stocks within a watershed such as the Fraser River basin (Dr. J. Woodey, Pacific Salmon Commission, Vancouver, B.C., personal communication). Nevertheless, the key premise is whether there are distinct isotopic differences in the natal sources of individual populations and how the isotopic signals from those differences can be distinguished. As compared with the tagging method, one of the greatest advantages is that the isotopic marker can trace the movement or migration of individual sockeye salmon between release and recovery and reconstruct the dynamic history of the stock.

It should be noted that for the techniques of stock identification, a recent development in trace elemental concentrations in otoliths has been extensively documented as "fingerprinting" (Campana et al. 1994). Multivariate analyses of the elemental fingerprints show success and potential in discrimination of cod stocks. Because of the theoretical uncertainty and technical difficulties in elemental analyses (Campana et al. 1997), stable isotope variations may be more useful than trace element methods as environmental tracers.

During sockeye salmon early life, the downstream and (or) seaward movement of smolts (Burgner 1991) occurred after age 1, with $\delta^{18}O$ values from –8.5 to –3.5‰. If we roughly defined –2.5‰ in $\delta^{18}O$ as the value of estuarine environments (Fig. 2), for instance, samples 23 and 22 were already living in the ocean, while sample 21 was still in the mouth of the Adams River system. The seaward migration of smolts and the rate of migrants thus could be potentially estimated from the isotope values of otoliths, although the migration pattern and speed were uncertain. In the Strait of Georgia, British Columbia, the distribution of sockeye salmon smolts is reported to be driven by the variability of winds, while tides and river discharges are of secondary importance (Peterman et al. 1994). The differences in temperature (warmer in nursery lakes and cooler in the estuary) and food sources between freshwater and marine settings might also play an important role in sockeye salmon early life (Figs. 2 and 3). Thus, the time differences of seaward migration for individual smolts were probably stimulated by both internal (e.g., age, growth, and heredity of the smolts; Wood et al. 1987) and external factors (e.g., temperature, salinity, and nutrients in the water; Burgner 1991). If the above isotopic interpretation and discussion are valid, it would be a very useful tool in determining the rate and proportion of seaward migrants.

It is quite surprising that during the marine stage of life history, both the $\delta^{18}O$ and $\delta^{13}C$ values of sockeye salmon otoliths are relatively uniform (Fig. 4). Previous studies concluded that sockeye salmon were found in a vast grazing area across the whole North Pacific between 46 and 60°N, where the summer surface temperature ranged from 5 to 16°C and salinity less than 32.2‰ (Foerster 1968; Burgner 1991). Because sockeye salmon are basically shallow-water swimmers (normally in the upper 20–30 m stratum), the overlap of isotope values of otoliths from different localities (Fig. 6) confirms that these sockeye salmon intermingle during their ocean lives and disperse as they return to their home streams after maturity. The isotopic interpretation is also consistent with evidence from tagging and biological studies (Foerster 1968). Furthermore, the average isotope variations of marine portions (ages 2–4) from different populations are also identical (Fig. 7). Since the distribution and abundance of sockeye salmon in the North Pacific varied from year to year and appeared to respond to climate conditions (Bisbal and McConnaha 1998), it is possible and potentially important to analyze the marine portion of sockeye salmon otoliths for long-term ecosystem changes or regime shifts as defined in Francis and Hare (1994) and Beamish et al. (1999). The gradual increase of $\delta^{13}C$ from 1995 to 1997 might suggest that there was a slow shift in pelagic plankton biomass (Fig. 7*a*). In contrast, the 0.6–0.8‰ shift in $\delta^{18}O$ around 1996 would be a sudden and significant change for the physical oceanic conditions (Fig. 7*b*). This phenomenon, if comparable, would correspond to a seawater temperature decrease of about 3–4°C (cf. Kalish 1991). To date, we have no direct evidence of a large-scale temperature change in 1996. However, the isotopic signal is an indication of how the thermal history of the ocean surface can be monitored in the otoliths of sockeye salmon and would be of interest in reconstruction of long-term ocean environmental changes.

In summary, we have demonstrated that the isotopic composition of otoliths is a promising chemical tracer in the study of sockeye salmon. Stable isotope ratio analysis (both $\delta^{18}O$ and $\delta^{13}C$) can be potentially used for (*i*) identification of the natal origin and distribution of sockeye salmon stocks in the northeast Pacific coast, (*ii*) determining the timing and rate of downstream and seaward migration of smolts, and (*iii*) studying the marine environmental and ecological changes as they pertain to climate-related regime shifts.

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Temperature and the recruitment of Atlantic cod (*Gadus morhua*)[1]

B. Planque and T. Frédou

Abstract: Variability in the recruitment of fish has been attributed to either changes in the environment or variations in the size of reproductive stocks. Disentangling the effects of environment and stock has proven to be problematic and has resulted in recurrent controversy between studies supporting either hypothesis. In the present study, we examine the relationship between interannual changes in temperature and variation in recruitment for nine Atlantic cod (*Gadus morhua*) stocks in the North Atlantic. We show that for individual stocks, the relationship often appears weak and statistically not significant. On the other hand, by combining in a single metaanalysis the results from individual stocks, we demonstrate that recruitment of Atlantic cod is linked to interannual fluctuations in temperature in such a way that for stocks located in warm water the relationship is negative, for stocks located in cold water the relationship is positive, and there is no relationship for stocks located in the middle of the temperature range.

Résumé : La variabilité du recrutement des populations nectoniques a été attribuée, tantôt aux changements de l'environnement, tantôt aux variations de la taille du stock reproducteur. La séparation des effets environnementaux de ceux liés aux stocks demeure problématique et a provoqué une contreverse entre les études appuyant l'une ou l'autre des deux hypothèses. Nous présentons ici, une étude des effets de la température sur le recrutement de neuf stocks de morue franche (*Gadus morhua*). Nous montrons que pour chaque stock pris séparément, la relation du recrutement avec la température apparait généralement faible et souvent non significative du point de vue statistique. En revanche, l'analyse combinée des neuf stocks permet de démontrer qu'une telle relation existe à l'échelle du bassin nord-Atlantique. Les variations du recrutement sont positivement liées aux changement de température dans les eaux froides, négativement dans les eaux chaudes et aucun lien entre le recruitment et la température n'est observé dans les eaux tempérées.

Introduction

Variability in fish recruitment has been attributed to either changes in the environment or variations in the size of reproductive stocks. Disentangling the effects of environment and spawning stock has proven to be problematic because of the small amount of data generally available, combined with a high degree of variability of this data. This has resulted in persistent controversy between studies supporting either hypothesis, particularly because of their implications for fisheries management strategies.

Biological research suggests that a number of environmental factors influence life history processes (e.g., see review by Brander 1997) and recruitment-related processes. For example, experimental work has demonstrated that temperature affects reproductive potential (Van Der Kraak and Pankhurst 1996), timing of spawning (Kjesbu 1994), embryonic and larval development (Rombough 1996), and growth (Solberg and Tilseth 1987). In addition, field observations have confirmed the effects of temperature on adult growth (Brander 1995), timing of spawning (Hutchings and Myers 1994*a*), and migration patterns (Rose et al. 1994). However, strong evidence of environmental control on recruitment operating in the wild is still sparse. Many of the environment–recruitment relationships proposed have been criticised for several reasons: first, because by scrutinising many environmental factors (exploratory analysis), one would ultimately find a relationship that is statistically significant but probably spurious and, second, because many of the relationships that were significant in first analysis have been rejected when retested with additional data (e.g., see Frank 1997 and Myers 1998). Yet, from these many attempts to relate recruitment variations to environment, one generalisation appears to stand out: in most cases, correlations for stocks located at the limit of the species' geographical distribution remain significant after retesting.

Atlantic cod (*Gadus morhua*) has provided one of the major North Atlantic fish resources, and its fisheries can be traced back to the very early days (see Kurlansky 1997). The species has been more intensively studied than any other marine fish species (Brander 1997). Cod stocks are found around the North Atlantic margin from Georges Bank to west of Greenland in the western North Atlantic and from

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Table 1. Summary of the data on Atlantic cod recruitment used in the present study.

Stock	Years	Age of recruits (years)	Mean bottom temperature (°C)
Newfoundland (NAFO 2J–3KL)	1959–1989	3	2
West Greenland (NAFO 1)	1952–1989	3	3
Northeast Arctic (ICES II)	1951–1990	3	4
Iceland (ICES Va)	1951–1993	3	5.8
Faeroes (ICES Vb)	1961–1994	2	7.4
Georges Bank (NAFO 5Z)	1977–1995	3	8
North Sea (ICES IV)	1960–1992	1	8.6
Irish Sea (ICES VIIa)	1968–1995	0	10
Celtic Sea (ICES VIIf–VIIk)	1970–1995	1	11

Note: Standard ICES and NAFO geographical areas for fisheries statistics are given in parentheses. Mean bottom temperatures are from Brander (1995).

the Celtic Sea to the Barents Sea in the eastern North Atlantic. The distribution of the species encompasses a wide range of temperature, with stocks distributed in waters with bottom temperature ranging from an average of 2 to 11°C (Brander 1995).

In the present study, we test the hypothesis that recruitment of Atlantic cod is affected by interannual changes in temperature in such a way that for stocks located in warm water the relationship is negative, for stocks located in cold water the relationship is positive, and there is no relationship for stocks located in the middle of the temperature range. A literature review on Atlantic cod stocks by Ottersen (1996), updated for the Irish Sea by Planque and Fox (1998), suggests that these effects of temperature can be detected for some stocks. However, the methods used to establish relationships between recruitment and temperature varied between studies, and for some stocks, distinct studies produced contradictory results. Also, there has not yet been a study that has analysed in a comparable way the relationship between temperature and recruitment across the North Atlantic.

Recently, the application of metaanalysis to the study of stock–recruitment relationships has demonstrated that the level of recruitment is related to the size of the reproductive stock, despite the apparent lack of statistical significance when stocks are considered independently (Myers and Barrowman 1996; Myers 1997). Here, using a similar strategy, we reexamine the temperature–recruitment relationship of nine Atlantic cod stocks and develop a method that combines the results from all individual stocks. By this approach, we can test for the existence of a gradual change in the nature of the temperature–recruitment relationship from cold to warm waters.

Materials and methods

It should be noted that the present work is not an exploratory study. Temperature was the only environmental parameter tested for correlation with recruitment. The selection of stocks, regions, and years of data was done on the basis of availability and known geographical distribution of stocks before performing the analysis.

Recruitment

Data on the number of recruits for nine stocks around the North Atlantic were taken from International Council for the Exploration of the Sea (ICES) or Northwest Atlantic Fisheries Organisation (NAFO) analyses and provided by R.A. Myers (see Myers et al. 1995 and internet site http://www.mscs.dal.ca/~myers/welcome.html).

Recruitment data were derived from virtual population analysis. In the virtual population analysis procedure, the number of recruits is back-calculated using data on the structure of the population measured by fisheries statistics and research surveys. We have always considered data on recruits for the youngest age available. The length of recruitment series and age of recruits varied among the nine stocks studied and are summarised in Table 1.

It is commonly accepted that recruitment is linked to the level of eggs spawned, which in turn is related to the size of the mature stock, generally referred to as the spawning stock biomass (SSB). The SSB fluctuates between years, and these fluctuations will contribute to changes in recruitment levels. Therefore, prior to studying the possible connection between environment and recruitment, it seems appropriate to correct recruitment data for SSB changes. This can be done by calculating the recruitment residuals from a fitted stock–recruitment model.

Although the existence of a relationship between stock and recruitment is evident from the theory (e.g., see Hilborn and Walters 1992) and established in practice (Myers and Barrowman 1996), determining the precise form of the stock–recruitment relationship in the case of individual stocks remains a difficult task. This is for several reasons. First, the data are generally highly scattered around the theoretical stock–recruitment curve. Second, the number of years of data is limited (rarely above 40) and the information on stock and recruitment at low values is rare, so that the shape of the curve close to the origin is not known precisely. Third, SSB–recruitment models such as Beverton–Holt or Ricker contain the implicit assumption of proportionality between SSB and egg production, but this assumption can be violated (see the case of Northeast Arctic cod in Marshall et al. 1998), so that the adequacy of the SSB–recruitment model can be questioned. In consequence, although in theory the level of recruitment is dependent on stock levels, there is in practice a large degree of uncertainty when estimating a particular model of the stock–recruitment relationship.

For each model that can be fitted to a specific stock, a time series of recruitment residuals can be calculated, and because a large range of models can be fitted, multiple series of recruitment residual can be derived. Of course, differences between these series are only due to the choice of the SSB–recruitment model and not to changes in recruitment itself, but when compared with environmental data, these different series may produce different results. In consequence, the choice of a particular fit of SSB–recruitment may corrupt the results of the analysis. For this reason, the recruitment series were not corrected for stock fluctuations. Instead, raw recruitment estimates were used for each stock studied. By doing this, it was assumed that the stocks have not reached such a low level that they cannot produce a strong year-class. However, be-

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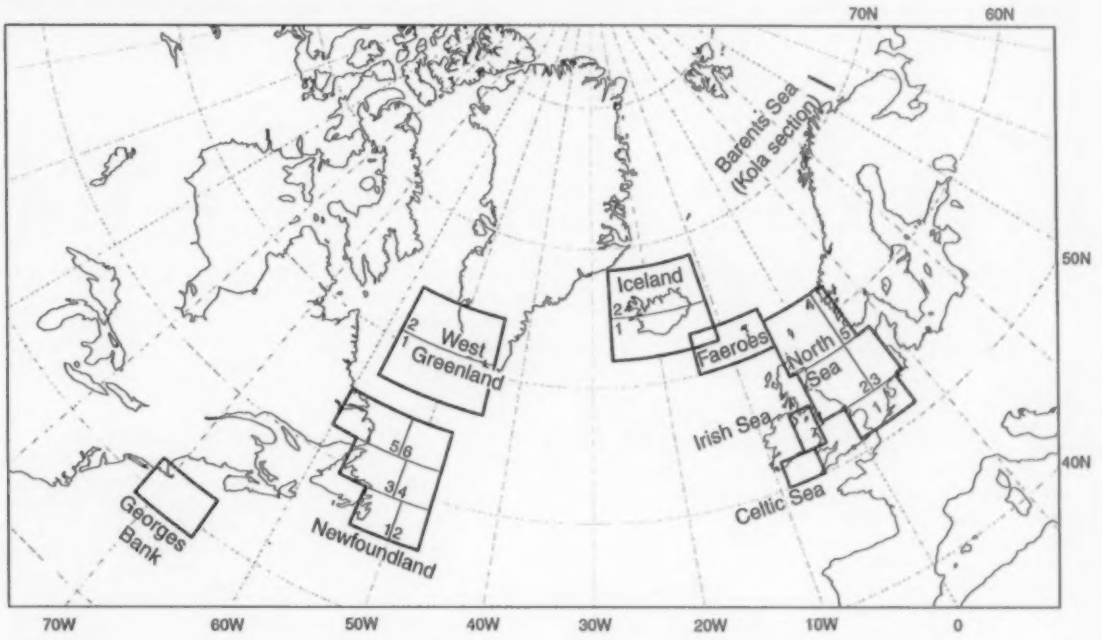
Fig. 1. Location of the areas and subareas for which SST series were selected. Areas are indicated by thick boxes and are named. Subareas are delimited by thin lines and are numbered.



cause Atlantic cod has been heavily exploited during the past decades, the above assumption can be violated in some cases. This will be discussed further.

Temperature

The temperature data suitable for comparison with Atlantic cod recruitment series have to fulfill several requirements. First, temperature time series have to be available for every stock. Second, the time series have to be consistent and sufficiently long to perform the analysis over a number of years. Third, the series will preferably correspond to large oceanic areas rather than to single sampling points so that they can be representative of the change in temperature experienced by the stocks over their area of distribution. This last point has been largely discussed in the case of the Northeast Arctic cod by Ottersen et al. (1998), who showed that environmental temperature does not strictly correspond to "ambient" temperature, although the population generally experiences higher temperature in warm years and vice versa.

A temperature data set that fulfills most of these requirements is the Comprehensive Ocean–Atmosphere Data Set (COADS) (Woodruff et al. 1987). COADS contains sea surface temperature (SST) in the form of monthly summaries in boxes of 2 × 2° and covers adequately the area of distribution of eight of the nine stocks studied. In the Barents Sea, the data available from COADS were too sparse and we used instead the temperature series of the Kola section (Tereshchenko 1996). The fluctuations in temperature of the Kola section and of other areas in the Barents Sea are almost synchronous, as shown by Ottersen et al. (1998) and by a comparison with temperature profiles from ICES (Frédou 1998), so that they are comparable with COADS data in other regions.

Temporal heterogeneity

Before comparing changes in recruitment with changes in temperature, it is necessary to define what is meant by "interannual variability in temperature." One of the most common ways to measure the interannual variability in temperature is to compare temperature values averaged over the months January–December. However, two years with similar annual temperature may display important seasonal differences (e.g., one year may have had a warmer summer that was compensated by a cooler winter). To assess how temperature anomalies are transferred from one month to the following ones, we have constructed the autocorrelation functions for temperature in each region. These functions indicate the degree of persistence of temperature anomalies.

First, the monthly temperature anomalies were derived from the original series as follows:

$$(1) \qquad a_{m,y} = t_{m,y} - t_m$$

where $a_{m,y}$ is the anomaly for a given month m and a given year y, $t_{m,y}$ is the original temperature record for the same month and year, and t_m is the temperature for month m averaged over all years. Second, the autocorrelation function (ACF) was calculated by comparing the original anomalies series with the same series lagged by increasing number of months. The ACF value calculated for each month is analogous to a correlation coefficient and varies from −1 (negative autocorrelation) to +1 (positive autocorrelation). The ACF was calculated for each region from lag 1 to 24 months, as in Wei (1990).

Spatial heterogeneity

In the same way that temperature anomalies can display temporal heterogeneity, these anomalies may show spatial heterogeneity at the subregional scale. When areas were large enough to perform the analysis, we assessed the degree of spatial heterogeneity by measuring the Pearson correlation coefficient between temperature anomalies time series in subareas. The values of the correlation coefficients are given as an indication of heterogeneity but were not tested for significance. Figure 1 indicates the areas and subareas for which temperature series were extracted. When comparing the

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Table 2. Correlation between temperature time series from distinct subareas in the North Sea, Iceland, West Greenland, and Newfoundland areas.

	North Sea						Newfoundland				
	1	2	3	4	Iceland, 1	West Greenland, 1	1	2	3	4	5
2	0.83				0.69	0.71	0.45				
3	0.89	0.92					0.65	0.16			
4	0.55	0.82	0.72				–0.02	0.20	0.06		
5	0.62	0.77	0.82	0.82			0.28	0.27	0.21	0.26	
6							–0.19	–0.03	–0.03	0.39	0.20

Note: The subareas are defined in Fig. 1. The temperature anomalies for each year are calculated over the months of February–June.

recruitment with temperature, we have always selected temperature averaged over the entire region (i.e., not the subareas).

Connection between temperature and recruitment for single stocks

When comparing recruitment and temperature, we have selected anomalies averaged over the months February–June, since this period encompasses the spawning season for all the stocks considered. The connection between temperature and recruitment was measured by correlation and regression analyses. The correlation analysis was used to test the statistical significance of the relationship for each stock, while the regression analysis was used to estimate the degree to which recruitment changes in relation to a 1°C temperature increase or decrease.

The recruitment estimates were $\log_2$ transformed prior to the correlation and regression analyses. The log transformation was used to stabilise the variance and to transform absolute changes in recruitment into relative changes so that all stocks are comparable despite large differences in mean recruitment levels. The correlation between interannual temperature changes and recruitment was determined for all stocks using the nonparametric Spearman rank correlation coefficient. One of the advantages of the nonparametric correlation over the standard parametric one is that the resulting coefficient is not influenced by outliers that are often present in biological data. Also, normality in temperature or recruitment data is not required to perform the analysis. Because autocorrelation exists in some of the recruitment and temperature time series, the significance of the correlation can be biased due to overestimation of the number of degrees of freedom. Therefore, we have reestimated the number of degrees of freedom using the general correction proposed by Quenouille (1952):

$$(2) \qquad N2 = N/(1 + r_1 + r_1' + r_2 + r_2' + \ldots)$$

where $N2$ is the adjusted sample size, N is the original sample size, r_1 and r_1' are the 1-year-lag autocorrelations of the two series, r_2 and r_2' are the 2-year-lag autocorrelations, and so on. The autocorrelations were calculated as in Wei (1990) and only the autocorrelation at 1-year lag was taken into account. The $N2$ can sometimes be greater than N (when autocorrelation is negative) and in such a case, the original sample size (N) was kept.

The regression analysis was performed using the method of Kendall and Gibbons (1990) given in Sokal and Rohlf (1995). As for the nonparametric correlation, the nonparametric regression does not require normality of the data and the slope of the regression curve is not affected by outliers. The connection between temperature changes and recruitment levels can be interpreted directly from the slope of the regression (β): a slope of +1 indicates that an increase of 1°C in temperature is associated with a twofold increase in recruitment, and, conversely a value of –1 shows that a similar temperature change is linked to a decline in recruitment by a factor of 2.

Connection between temperature and recruitment for combined stocks

In addition to analyses of temperature–recruitment relationship for each individual stock, we have studied the temperature–recruitment relationship across all Atlantic cod stocks in a single analysis. In this approach, one does not test the significance of single relationships but rather whether the relationship between recruitment and temperature varies from one end of the temperature distribution of Atlantic cod to the other. Here, we hypothesise that the relationship is positive at the cold limit of the temperature distribution and negative at the warm limit. It is also expected that for stocks not located at the edge of the temperature distribution, there will be no relationship between temperature and recruitment. To test this hypothesis, we have used the slope of the regression line β as a measure of the temperature–recruitment relationship and have tested whether this slope varies from being positive in cold waters to being negative in warm ones. The uncertainty in the regression slope β was assessed by estimating the empirical distribution of β using a bootstrapping procedure (Efron 1979). This was performed by resampling the residuals from the regression 250 times for each stock. Then the Spearman rank correlation coefficient between empirical distributions of β and mean bottom temperatures was calculated. To do so, we ranked the 250 values of each distribution and subsampled 10% of the values at regular intervals. The aim of that procedure was to reduce the number of data in each distribution from 250 to 25 for computational purposes (we later tested that this had no detectable effect on the final correlation value). The Spearman rank correlation was calculated on the resulting 225 values (25 times nine stocks). The coefficient was tested against its empirical distribution estimated by a Monte-Carlo permutation procedure (5000 permutations). The permutation was performed on the mean bottom temperature in such a way that each empirical distribution was kept unchanged but the associated bottom temperature was permuted (i.e., there was no permutation of individual slope values between distributions). It should be noted that because we have a prior expectation on the nature of the alternative hypothesis (β should decrease with increasing temperature), the statistical test is one-sided. Therefore, the p values used are also one-sided.

Results

Spatial and temporal heterogeneity in regional temperatures

Spatial heterogeneity in temperature series was assessed for regions covering sufficiently large oceanic areas: North Sea, Icelandic Sea, West Greenland, and Newfoundland waters. Table 2 gives the Pearson correlation coefficients between subareas. These coefficients indicate the degree of similarity in temperature time series within regions. In the North Sea, all the correlation values are positive and high, indicating that interannual temperature changes in the differ-

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Fig. 2. ACF for the time series of monthly temperature anomalies for the nine areas in the North Atlantic. The value in parentheses is the mean autocorrelation value from lag 1 to 4 months (indicated by the solid circles) and is an indicator of the persistence of temperature anomalies over 5-month periods (such as the February–June period selected for comparison with recruitment data).

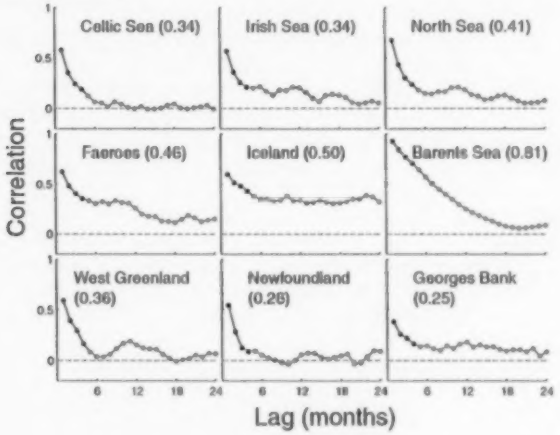

Fig. 3. Scatterplots of $\log_2$ recruitment of Atlantic cod against temperature (February–June) for the nine stocks in the North Atlantic. The lines correspond to the fit of a nonparametric regression (see Materials and methods section for details). Note that recruitment anomalies for West Greenland are plotted on a distinct scale due to the higher variability in recruitment in this region.

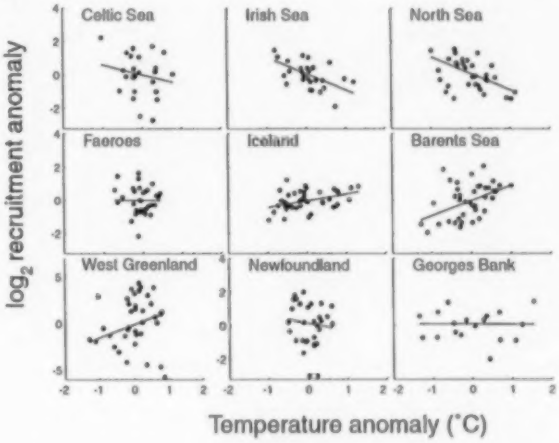

Table 3. Spearman correlation coefficient (r) and regression slope (β) between temperature and $\log_2$ recruitment of Atlantic cod.

Stock	r	β	N	$N2$
Newfoundland	–0.13 ns	–0.43	31	(33)
West Greenland	0.23 ns	1.43	38	31
Northeast Arctic	0.42*	0.92	40	31
Iceland	0.38*	0.41	43	35
Faeroes	–0.05 ns	–0.02	34	26
Georges Bank	–0.08 ns	–0.01	18	18
North Sea	–0.42*	–0.98	33	32
Irish Sea	–0.54**	–0.98	27	(29)
Celtic Sea	–0.18 ns	–0.58	25	21

Note: ns, not significant; *$p < 0.05$; **$p < 0.01$. N is the sample size for each stock and $N2$ is the adjusted sample size estimated from the method of Quenouille (1952) (see Materials and methods section for details). Parentheses in the $N2$ column indicate when $N2$ is greater than N.

ent subareas have followed parallel patterns. For Iceland and West Greenland, only two subareas were compared and in both cases the correlations are positive and high. In Newfoundland, correlation values between the five subareas selected are much lower, often close to zero and sometimes negative. This indicates that interannual fluctuations in temperature vary considerably within the Newfoundland area. It also suggests that in this region, the choice of area over which the temperature is averaged is critical to the resulting temperature time series.

Temporal heterogeneity was assessed for all regions. Figure 2 shows the ACFs based on monthly temperature anomalies in each region. For each area, the ACF starts with a positive value and decreases towards zero. The first four lags are highlighted in the figure, as they correspond to the persistence of a temperature anomaly for a period of 5 months, equivalent to the February–June period chosen for comparison between temperature and recruitment (see next section). The average correlation over the four lags is also indicated as an index of persistence of the temperature anomaly. For all regions, the ACFs show a marked decline during the first 4 months, but the level of correlation varies between stocks. In the Barents Sea, the correlation at lag 1 month is the highest ($ACF_1 = 0.93$) and it remains the highest when integrated over 4 months ($ACF_{1\text{-}4} = 0.81$). This indicates that the interannual variability in temperature is likely to remain similar, even if based on a different set of months (warm winters generally coincide with warm springs, etc.). In the case of the Newfoundland region, the autocorrelation displays a sharp decrease and is already close to zero at lag 3 months. In contrast with the Barents Sea, in the Newfoundland region the choice of the months retained to calculate interannual changes in temperature is critical. The ACFs for the other regions display various patterns between the two extremes. It can be noted that the mean autocorrelation values for the first four lags are generally higher in the eastern than in the western part of the North Atlantic.

Regional connections between temperature and recruitment

Relationships between SST and Atlantic cod recruitment for the nine stocks have been assessed using Spearman's rank correlation. Table 3 shows the correlation coefficient, original and corrected sample size, and slope of the nonparametric regression for each stock. Figure 3 shows $\log_2$ recruitment anomalies plotted against temperature anomalies for each stock together with the linear regression fit.

In West Greenland, recruitment varied by up to a factor of 1000 with temperature fluctuations of about 2°C. In Newfoundland and Faeroes, the amplitude of changes in temperature is lower (<1°C) and recruitment varied by up to a

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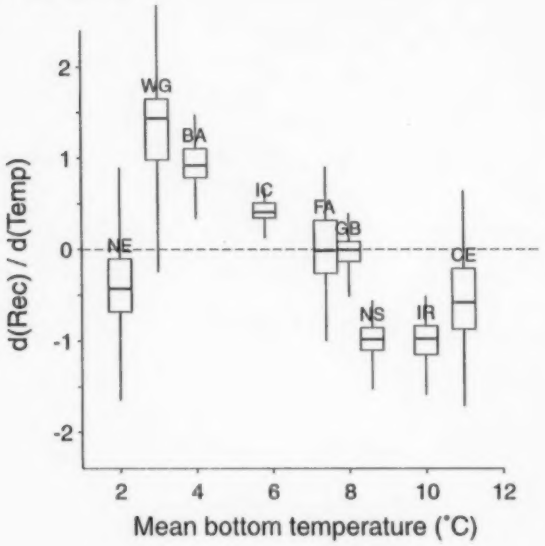
Fig. 4. Link between SST and recruitment plotted against mean bottom temperature for nine Atlantic cod stocks in the North Atlantic. For each stock, the link is estimated by the slope of the nonparametric regression (β from Table 3) represented by the horizontal line within the box. Boxes and lines indicate the 50 and 95% limits of the empirical distribution of the slopes, determined by a bootstraping procedure. Stocks: NE, Newfoundland (northern cod); WG, West Greenland; BA, Barents Sea (Northeast Arctic cod); IC, Iceland; FA, Faeroe Islands (Faeroes); GB, Georges Bank; NS, North Sea; IR, Irish Sea; CE, Celtic Sea.



factor of 30. The stock of Georges Bank shows similar variations in recruitment despite a larger range in temperature variation (about 4°C). These results show that interannual changes in recruitment are generally large and that the amplitude of these changes varies among stocks. The data are also highly scattered around the regression lines, and the statistical significance of the relationships is generally low (Table 3). In the Celtic Sea, Faeroes, West Greenland, Georges Bank, and Newfoundland, correlations are not significant. Correlations are significant in the North Sea, Barents Sea, and Iceland at $p < 0.05$ and in the Irish Sea at $p < 0.01$.

Positive relationships (increase in Atlantic cod recruitment at high temperature anomalies) are significant in the Barents Sea and Iceland, whereas negative relationships are significant in the Irish Sea and North Sea. However, we are left with the observation that for the Celtic Sea and Georges Bank stocks (stocks located at the warm limit) and for the West Greenland and Newfoundland stocks (cold limit) the correlation analysis cannot reject the null hypothesis that no relationship between temperature and recruitment exists. Therefore, it is difficult to conclude from the correlation results alone that a gradual change in the temperature–recruitment relationship does exist.

The conclusions of the stock-by-stock correlation analysis are that (*i*) there is a high degree of scatter in the data that results in difficulty in obtaining statistically significant relationships and (*ii*) for stocks located at the cold and warm temperature limits, it is often not possible to conclude that a relationship between temperature and recruitment exists. One way to overcome the limitation of the above correlation analysis is to combine the data from all stocks in a single transatlantic analysis.

Transatlantic connection

Results from all stocks were combined in a single analysis and the gradual change in the nature of the temperature–recruitment relationship was assessed by testing the existence of a link between the regression line slope β and the mean bottom temperature of each stock. Figure 4 shows the distributions of β against the mean bottom temperatures. The apparent trend is for β to be positive for stocks located in cold waters and gradually decrease to negative values for stocks in warm waters. For temperate waters the value of β is close to zero, as expected. The rank correlation between β distributions and mean bottom temperature is $r = -0.59$ and the associated probability is $p = 0.027$. This indicates that the null hypothesis (effect of temperature on recruitment is independent of mean temperature) can be rejected. It is clear, however, from Fig. 4 that the northern cod stock (Newfoundland) stands out of this general pattern.

When performing the calculation of β, several assumptions were made: (*i*) the area over which temperature is averaged is representative of the area of distribution of the Atlantic cod stock, (*ii*) the temperature anomaly signal does not vary substantially within the 5-month period over which it is averaged, and (*iii*) recruitment has not been affected by exceptional forcing that would have masked the effects of temperature. In the case of the northern cod stock, these three assumptions are violated. First, the spatial homogeneity in temperature in this area is extremely low, as shown by the correlations in Table 2. Second, there is a high degree of intermonth variability, as demonstrated in Fig. 2. Third, it is reasonable to assume that the effect of temperature on the recruitment of Northern cod have been masked by the dramatic reduction in SSB that preceded the collapse of the stock (Hutchings and Myers 1994*b*; Myers et al. 1997). In consequence, the estimate of β for the northern cod stock is highly uncertain and it would be appropriate to treat this stock separately from the others. When the northern cod stock is removed from the analysis, the rank correlation between β distributions and mean bottom temperature reaches $r = -0.83$ and the statistical significance is greatly improved with $p = 0.0016$.

Discussion

In their demonstration of the relationship between stock and recruitment levels, Myers and Barrowman (1996) illustrated how powerful metaanalysis can be when single analyses fail to give significant answers. Later, Myers (1998) recommended that general hypotheses on recruitment should be tested with a similar approach, using data sets from several populations. In this study, we present a metaanalysis of data on nine cod stocks and show that the relationship between temperature and recruitment follows a gradual change: a positive relationship in cold waters, no relation-

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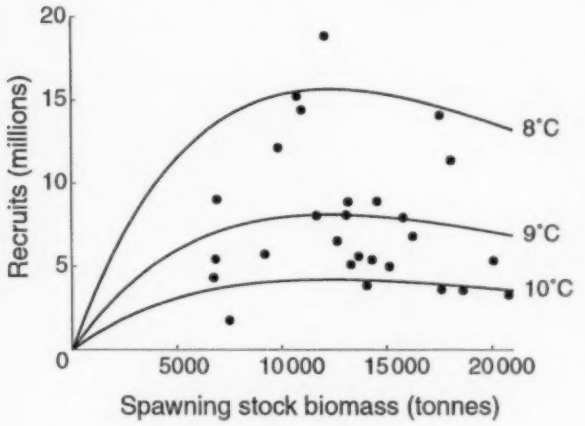
Fig. 5. Recruitment of Irish Sea cod plotted against SSB (points). The lines show the fit of a combined stock/temperature–recruitment model for three distinct temperatures. The model (eq. 4) was fitted using least squares nonlinear regression.



ship in temperate waters, and a negative relationship in warm waters. The present study demonstrates the existence of a significant temperature–recruitment relationship despite the general pattern of apparent low (or lack of) statistical significance of correlation analyses on single stocks. This transatlantic relationship is consistent with the view that the effects of the environment on recruitment should be stronger at the limit of a species' spatial distribution. It can be seen as a unifying factor for previous results from a number of studies that showed apparently contradictory results on the effects of temperature on Atlantic cod recruitment.

It has been argued that temperature may not be directly linked to recruitment but only acts as a proxy for marine productivity in lower trophic levels (Rothschild 1994). In the present study, the link between temperature and recruitment is only evident at the limit of Atlantic cod distribution, which suggests that direct effects of temperature on recruitment could exist independently of food-related control.

There are immediate applications that could be made of such knowledge. For example, monitoring SST is a relatively easy task that could help in forecasting recruitment levels several months in advance. Such short-term forecasting is probably of limited value because assessment surveys already produce recruitment estimates within a few months, but it could be used as a test for the accuracy of the relationship. Also, discrepancies between temperature-based forecasts and actual surveys should raise questions about possible changes in the population dynamics, the nature of environmental forcing, or the accuracy of recruitment estimates. For example, taking into account the link between temperature and recruitment could explain the unexpected strong year-class of North Sea cod in 1996, which was associated with the rapid and extreme cooling of the North Sea (Loewe 1996) and the following poor year-class of 1997 when warmer conditions prevailed.

One can also envisage medium- to long-term forecasting of recruitment based on climate forecasts. Although such prediction would certainly be of great value to fisheries management, this idea finds its limits in the low accuracy of medium-range (10 years) climate predictions upon which the recruitment forecasts would depend.

In short- and long-term forecasts, it has to be remembered that the fluctuations in temperature only account for a limited fraction of the recruitment variability in an individual stock, so that the uncertainty associated with any prediction will be high. But forecasting recruitment is not the only way by which temperature information can be used for fisheries management.

The stock–recruitment relationship is the core of current management procedures and is used to determine the level of fishing mortality that a stock can support and ultimately to advise on total allowable catch. Yet, most of the stock–recruitment models do not currently include environmental factors when this could be easily achieved. For example, one can build a stock–recruitment model from the Ricker model (e.g., see Hilborn and Walters 1992):

$$R = a \cdot \mathrm{SSB} \cdot e^{-\beta \cdot \mathrm{SSB}} \quad (3)$$

(or its equivalent form $R = \mathrm{SSB} \cdot e^{(\alpha - \beta \cdot \mathrm{SSB})}$)

and include temperature in a form similar to that proposed by Stocker et al. (1985):

$$R = \mathrm{SSB} \cdot e^{(\alpha - \beta \cdot \mathrm{SSB})} \cdot e^{(\Psi \cdot T)} \quad (4)$$

where R, SSB, and T are recruitment, spawning stock biomass, and temperature, respectively, and α, β, and Ψ are the associated coefficients. The resulting model is a three-dimensional surface (where SSB, T, and R are the three dimensions). Figure 5 shows an example of such a fit for the Irish Sea cod stock. The modelled "surface" is plotted in the form of three curves corresponding to three temperatures "slices" within the range of observed values in the Irish Sea. It is clear that the fitted curves and particularly the slopes at the origin vary greatly under the distinct temperature regimes. Such information could be used to assess the impact of management strategies under various climatic scenarios.

Biological research is needed to confirm the temperature–recruitment links described here and to understand the underlying mechanisms. This is particularly important for two reasons. First, it is unlikely that temperature–recruitment relationships will ever be used in fisheries management if the mechanism by which temperature modulate recruitment cannot be demonstrated. Second, if one can understand such mechanisms and identify which key processes are affected by temperature changes, it might be possible to design management strategies that will tend to maximise the recruitment chances of a given population on a biological basis.

It has recently been observed that the changes in population structure and particularly the reduction in age-class distribution are associated with the fluctuations in recruitment (Marteinsdottir and Thorarinson 1998). In addition, Kjesbu et al. (1996) have showed that the intensity of spawning and the extent of the spawning period vary with the size of individual Atlantic cod. From this observation, Ottersen et al. (1994) have argued that populations with reduced age composition (and consequently reduced size composition) would spawn over a smaller geographical area and for a shorter time period and might therefore become more sensitive to

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changing environmental conditions. If such is the case, it is probable that in the near future, most of the heavily exploited fish populations will display amplified responses to environmental changes.

Understanding the effects of environment on recruitment could be of great value if integrated into management procedures, and the possible ways by which this could be done have not yet been fully explored. The main difficulty will likely be in reconciling results on temperature–recruitment relationships that are evident at the transatlantic scale and management strategies that take place at the level of individual stocks only.

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Water quality requirements of smolting Atlantic salmon (*Salmo salar*) in limed acid rivers

Frode Kroglund and Magne Staurnes

Abstract: Groups of Atlantic salmon (*Salmo salar*) smolts were held 1–13 days in soft water with a pH range of 5.0–6.6, concentrations of labile inorganic monomeric Al (Al_i) of 10–90 $\mu g \cdot L^{-1}$, and 0.7–2.3 mg $Ca \cdot L^{-1}$. Fish were exposed to either naturally acidic water from a river in southwestern Norway, limed water from the same river, mixtures of acidic and limed river water, acidic river water with sulfuric acid and Al added, or limed river water with additional lime. Mortality was observed in all groups exposed to water with pH < 5.8 and containing 30–90 μg $Al_i \cdot L^{-1}$. No fish died in water with pH > 5.8 and 15–20 μg $Al_i \cdot L^{-1}$, but fish in water with pH 5.8–6.2 had lower plasma Cl^- concentration and gill Na^+,K^+-ATPase activity than fish in water with pH > 6.5. Smolts exposed to pH < 5.8 were unable to survive in seawater, and smolts exposed to water with pH 5.8–6.2 had lower hypoosmoregulatory capacity than smolts exposed to water with pH > 6.5. These results show that even moderately acidified water with low Al concentrations impairs smoltification and reduces the seawater tolerance of Atlantic salmon smolts.

Résumé : Des groupes de saumoneaux du saumon de l'Atlantique ont été placés pendant de 1 à 13 jours dans de l'eau douce dont le pH variait de 5,0 à 6,6 et la concentration d'aluminium monomère inorganique labile (Al_i) de 10 à 90 μg $Al_i \cdot L^{-1}$ et celle du Ca de 0,7 à 2,3 $mg \cdot L^{-1}$. Les poissons ont été exposés à l'eau naturellement acide d'une rivière du sud-ouest de la Norvège, à de l'eau chaulée de cette même rivière, à des mélanges d'eau de la rivière acidifiée et chaulée, à l'eau acide avec ajout d'acide sulfurique et de Al ou à l'eau chaulée avec ajout de chaux. Des décès ont été notés chez tous les groupes exposés à de l'eau de pH < 5,8 et contenant de 30 à 90 μg $Al_i \cdot L^{-1}$. Aucun poisson n'est mort dans de l'eau à pH > 5,8 et à concentration d'aluminium de 15-20 μg $Al_i \cdot L^{-1}$, mais les poissons se trouvant dans l'eau à pH de 5,8 à 6,2 présentaient des concentrations de Cl^- plasmatique inférieures et une activité de la Na^+,K^+-ATPase des branchies inférieure à celle des poissons placés dans l'eau à pH > 6,5. Les saumoneaux exposés à de l'eau de pH < 5,8 étaient incapables de survivre en eau de mer et ceux exposés à de l'eau à pH de 5,8 à 6,2 présentaient une capacité hypo-osmorégulatrice inférieure à celle des saumoneaux exposés à de l'eau de pH > 6,5. Ces résultats montrent que de l'eau même modérément acide et présentant de faibles concentrations de Al nuit à la smoltification et réduit la tolérance à l'eau de mer des saumoneaux du saumon de l'Atlantique.

[Traduit par la Rédaction]

Introduction

Acidification has caused severe damage to freshwater ecosystems over large areas of Europe and North America (e.g., Muniz 1991). The Atlantic salmon (*Salmo salar*) is the most sensitive of the naturally occurring salmonids in Europe (Rosseland and Skogheim 1984; Exley and Phillips 1988). In southern Norway, acidification has completely eliminated a number of salmon stocks and severely diminished others (e.g., Hesthagen and Hansen 1991).

Atlantic salmon are more sensitive to acidic water during the parr–smolt transformation than at other life stages (e.g., Rosseland and Skogheim 1984; Staurnes et al. 1993*a*). The exposure of smolting Atlantic salmon to acidic water has been found to cause ionoregulatory failure in freshwater, inhibit the increase in gill Na^+,K^+-ATPase activity, and reduce seawater tolerance (e.g., Farmer et al. 1989; Staurnes et al. 1993*a*). The presence of even low concentrations of Al in the water greatly enhances the toxicity of acidic water (Staurnes et al. 1993*a*). In soft water low in organic matter, which is typical of acidified areas in Scandinavia, H^+ and labile inorganic monomeric species of Al are, together with Ca, considered to be most important in determining the toxicity of acidic water (e.g., Exley and Phillips 1988; Rosseland et al. 1990; Rosseland and Staurnes 1994). Because of the increased sensitivity to acidic water that occurs when the parr develop into fully seawater-tolerant smolts, it has been suggested that water qualities not normally regarded as toxic to salmonids may be so for smolts and may thus contribute to the reduction of salmon stocks even in rivers that are not chronically acidic and are not normally regarded as being in danger of acidification (Staurnes et al. 1993*a*, 1995).

In the 1970s and 1980s, a marked decrease in salmon catches and recruitment failure of salmon were recorded in rivers in southwestern and western Norway (Kroglund et al. 1994). One of these was the River Vikedalselva. In the early 1980s, fish kills were recorded annually after spring episodes characterized by reduced pH and elevated concentrations of Al. This provided evidence that the river was seriously affected by acid rain (Henriksen et al. 1984;

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Table 1. Water quality parameters and Atlantic salmon smolt exposure groups in 1991.

Water	Group	pH, mean (SD)	Al_a, $\mu g \cdot L^{-1}$, mean (SD)	Al_i, $\mu g \cdot L^{-1}$, mean (SD)	Ca, $mg \cdot L^{-1}$, mean (SD)
Limed river water (LRW)	Reference, limed	6.24 (0.08)	24 (5)	18 (5)	1.33 (0.08)
Acid river water (ARW)	Acid	5.54 (0.07)	31 (6)	29 (6)	0.72 (0.03)
Mixed 60% LRW and 40% ARW water	Mixed pH 6.12	6.12 (0.11)	19 (14)	11 (8)	1.10 (0.08)
Mixed 40% LRW and 60% ARW water	Mixed pH 6.06	6.06 (0.04)	26 (4)	17 (6)	1.00 (0.06)
Mixed 15% LRW and 85% ARW	Mixed pH 5.78	5.78 (0.06)	31 (7)	28 (5)	0.81 (0.03)
ARW with acid and 40 μg $Al \cdot L^{-1}$ added	Moderate Al	5.22 (0.06)	75 (7)	60 (5)	0.73 (0.04)
ARW with acid and 80 μg $Al \cdot L^{-1}$ added	High Al	5.05 (0.03)	109 (8)	91 (7)	0.74 (0.04)

Note: Values of water parameters are means of water samples taken every day or every second day during the experimental period ($N = 6$). The reference group consists of undisturbed fish held in limed river water in the storage tanks indoors. All other groups were held outdoors in 90-L tanks supplied with water as described.

Hesthagen 1989). Salmon smolts was the life stage most seriously affected during these episodes. Even though the river has been limed since 1987, liming has not brought about the expected increase in the salmon stock (Kroglund et al. 1994). The river is limed so that the pH is 6.2 during the smolting period. Because of the slow increase in the salmon stock following the start of liming, we questioned whether the water quality in the river had actually improved sufficiently to ensure survival of smolts. Therefore, the main objective of our experiment was to study the effects of moderately acidic water containing low concentrations of inorganic monomeric Al (Al_i) on osmoregulation and seawater tolerance of Atlantic salmon smolts. We also tested for detrimental effects on smolts of short-term exposures to acidic water, simulating operational failures in the automatic lime doser.

Materials and methods

Study site

The exposure experiments were conducted in April–May 1991 and 1992 about 700 m downstream of the automatic lime doser located at the Låkafossen waterfall on the River Vikedalselva in southwestern Norway. The width of this river ranges from 20 to 30 m, and the annual mean discharge at the river mouth is 9.0 $m^3 \cdot s^{-1}$. Anadromous salmonids ascend 10 km up the river system as far as the Låkafossen waterfall (Hesthagen 1989). Mean values of chemical parameters from water samples from above Låkafossen in the period 1980–1990 were pH 5.44, 50 μg acid-reactive Al (Al_a)$\cdot L^{-1}$, 18 μg $Al_i \cdot L^{-1}$, 0.73 mg $Ca^{2+} \cdot L^{-1}$, 0.37 mg $Mg^{2+} \cdot L^{-1}$, 0.21 mg $Na^{+} \cdot L^{-1}$, 4.04 mg $Cl^{-} \cdot L^{-1}$, 2.42 mg $SO_4^{2-} \cdot L^{-1}$, 170 μg $NO_3^{-} \cdot L^{-1}$, conductivity 24.9 $\mu S \cdot cm^{-1}$, alkalinity 2.4 $\mu equiv. \cdot L^{-1}$, and total organic content 1.14 mg $C \cdot L^{-1}$ ($N = 176$, except $N = 126$ for Al_a and Al_i) (Hindar and Henriksen 1992). The dissolved organic content corresponded to about 90% of the TOC value.

Because of the annual kills of smolts during spring acidification episodes from the early 1980s, an automatic lime doser was placed at Låkafossen in 1987 to increase the pH to 6.2 with lime slurry during the 1 March – 31 May period (Hindar and Henriksen 1992). An automatic pH logger installed in 1987 showed episodic low pH values (< 5.5) during the summer, fall, and winter, so since 1989 the river has been limed to produce a pH of 6.2 from 15 February to 1 June and a pH of 5.7 for the rest of the year. Fish kills have not been reported since 1987, probably as a result of the liming (Kroglund et al. 1994).

Experimental fish

The experiments were carried out using 1-year-old, first-generation hatchery-reared Atlantic salmon smolts (25–40 g, 13–17 cm) of River Vikedalselva (1991) or River Imsa stocks (1992). The fish were reared at the Ims Research Station in southwestern Norway in circumneutral water with a low concentration of Al_i (see Table 2).

The fish were transported to the experimental site in tanks with oxygenated freshwater on 2 May 1991 and 22 April 1992 (transport distance about 50 km, time about 4 h) and thereafter transferred to two 1-m^2 EWOS storage tanks indoors, supplied with limed river water. At the time of transport, the fish had a more complete smolt coloration in 1991 than in 1992 (more silvered, no visible parr marks, darker fin margins). In 1992, the fish had slightly visible parr marks.

Water qualities

The exposure experiments were conducted outdoors in 90-L black circular plastic tanks (water volume 60 L) continuously supplied with water of different qualities (Tables 1 and 2; Fig. 1): acidic river water from upstream of the lime doser, limed river water from 700 m downstream of the doser, mixtures of acidic and limed river water, acidic river water with both sulfuric acid and Al as $AlCl_2 \cdot H_2O$ added (1991 only), mixed limed and acidic river water with sulfuric acid added (1992 only), and limed river water with lime slurry added (1992 only).

The water mixtures were produced in large header tanks to allow aging of the water to avoid the effects of "mixing zone" water chemistry in the exposure tanks. When acidic water mixes with less acidic or limed water, it creates a zone of unstable Al chemistry where Al_i goes from low to high molecular forms and polymerizes (Rosseland et al. 1992). The residence time in the header tanks was about 15 min in 1991 and 90 min (tank with lime slurry added) or 75 min (all other tanks) in 1992. The residence time in the 90-L exposure tanks was about 15 min in 1991 and 30 min in 1992.

On 19 April 1992, 3 days before the fish were to be transported, a failure in the lime doser caused a greater variation in the limed river water quality than in 1991. Lime was released in accordance with an aberrant signal that indicated pH 3.0 downstream of the lime doser, resulting in a marked overdosing of the river. The mean pH from 23 April until the pH gradually had returned to the normal value of about 6.2 on 1 May was 6.5 (Table 2; Fig. 1).

Experimental design in 1991

After transport on 2 May the fish were held for 7 days in limed river water in two indoor storage tanks. Some fish were held undisturbed in these tanks until the end of the experiment on 14 May (reference group). Groups of about 100 experimental fish were transferred on 9 May to each of seven exposure tanks supplied with water of different qualities (Table 1) (temperature 10–12°C, water flow about 4 $L \cdot min^{-1}$, biomass about 3 kg).

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Table 2. Water quality parameters and Atlantic salmon smolt exposure groups in 1992.

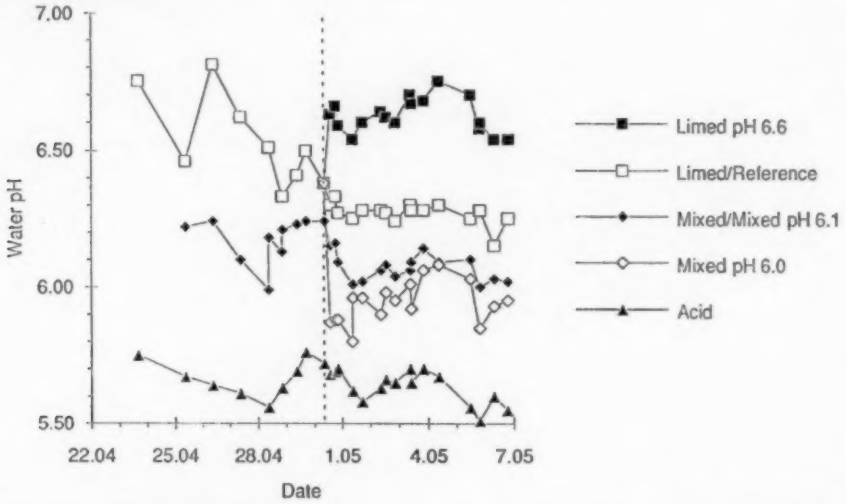
Water	Group	Period	pH, mean (SD)	Al_a, $\mu g \cdot L^{-1}$, mean (SD)	Al_i, $\mu g \cdot L^{-1}$, mean (SD)	Ca, $mg \cdot L^{-1}$, mean (SD)
Imsa water	Control	—	6.8	15	<10	3.3
Limed river water (LRW)	Reference, limed, 24 h limed*	23–30 April	6.52 (0.14)	43 (8)	22 (16)	2.27 (0.64)
		1–6 May	6.28 (0.06)	39 (4)	15 (2)	1.64 (0.34)
Acid river water (ARW)	Acid, 24 h acid*	23 April – 6 May	5.63 (0.07)	48 (8)	35 (12)	0.65 (0.06)
Mixed LRW and ARW water (MRW)	Mixed, mixed pH 6.1†	23–30 April	6.16 (0.10)	42 (8)	13 (2)	2.12 (0.74)
		1–6 May	6.07 (0.07)	35 (3)	18 (2)	1.17 (0.14)
LRW with lime slurry added	Limed pH 6.6†	1–6 May	6.57 (0.11)	36 (4)	15 (6)	2.19 (0.71)
MRW with acid added	Mixed pH 6.0†, 24 h mixed*	1–6 May	5.95(0.10)	37 (6)	15 (5)	0.96 (0.10)

Note: Values for the River Imsa are the normal values of the rearing water at the Imsa Research Station (only small variation in water quality). Values for water quality parameters are means of water samples taken every day or every second day during the experimental period (23–30 April, $N = 5$; 1–6 May, $N = 8$). The parameters of the limed river water (and therefore also of the mixed limed and acidic river water) differed during the period 23 April – 1 May from the normal values from 1 May and the rest of the experimental period due to a failure in the lime doser (see Fig. 1). See text for further explanation of experimental design.

*From 2 to 3 May.

†From 1 May.

Fig. 1. Fluctuations in pH in the different water qualities during the experimental period in 1992. The limed pH 6.6, mixed pH 6.1, and mixed pH 6.0 Atlantic salmon smolt groups experienced the same water quality as the limed group until 30 April (indicated by the vertical line), when the water qualities were changed to those indicated. See text and Table 2 for further explanation of water quality manipulations and group notations.



Mortality was recorded two or three times a day. At the end of the experiment on 14 May, 20 fish from each of the exposure groups with surving fish were transferred to 90-L exposure tanks containing 40 L of 35 ppt aerated seawater (temperature 10–13°C). We used artificial seawater made by adding artificial "instant seawater" salt to freshwater 2 days before the start of the seawater challenge test. Mortality was recorded after 24 h. A group of 20 reference fish was also transferred to seawater to study possible effects of the exposure procedure on seawater tolerance.

Experimental design in 1992

The 1992 experimental design varied from that in 1991 to minimize handling of the fish, increase exposure time, identify sublethal effects in water of moderate acidity, and study the possible effects of short-term exposures to moderately acidic water.

After transport, fish were transfered to the two indoor storage tanks on 22 April. Some fish were held in these tanks until the end of the experiment on 6 May (reference group). The next day, 60 experimental fish were placed in each of nine exposure tanks (Table 2) (temperature 6–7°C, water flow about 2 $L \cdot min^{-1}$, biomass about 1.8 kg). Three of the tanks received either limed river water (limed group), acidic river water (acid group), or mixed acidic and limed river water (mixed group) during the whole expeimental period. Three other tanks received limed river water until 1 May. The water supply was then changed to either limed river water with lime slurry added (limed pH 6.6 group), mixed water as in the mixed group (mixed pH 6.1 group), or mixed water with acid added (mixed pH 6.0 group). These water supplies were unchanged until the end of the experiment on 6 May.

The fish in the remaining three tanks were used to study possible effects of short-term exposures to moderately acidic water (during a short-term failure in the lime doser). The tanks were supplied

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Table 3. Hematocrit, blood plasma Cl^- concentration, and gill Na^+,K^+-ATPase in different groups of Atlantic salmon smolts in 1992 (see text and Table 2 for explanation).

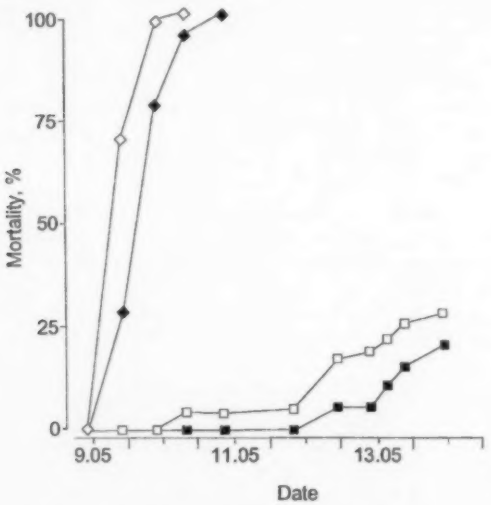
Group	Sampling date	Exposure time, days	*N*	Hematocrit, %, mean (SD)	Plasma Cl^-, mM, mean (SD)	Na^+,K^+-ATPase, μmol P_i·mg protein^{-1}·h^{-1}, mean (SD)*
Control	5 May	—	8	45.5 (3.3)	128.6 (1.1)	5.60 (1.78)
Limed	24 April	1	7	40.4 (3.5)	110.7 *d* (7.3)	—
Mixed	24 April	1	3	46.0 (3.6)	112.5 *d* (7.3)	—
Acid	24 April	1	3	45.7 (3.5)	104.8 *d* (7.5)	—
Limed	29 April	6	12	38.8 (2.3)	126.8 (4.9)	—
Mixed	29 April	6	8	47.5 (3.3)	103.1 *bd* (17.7)	—
Reference	6 May	13	12	44.3 (4.0)	122.3 *cd* (3.9)	—
Limed	6 May	13	19	43.5 (4.3)	122.8 *cd* (6.6)	4.43 *c* (2.04)
Mixed	6 May	13	23	40.9 (3.6)	118.3 *bcd* (4.7)	3.93 *cd* (0.78)
Acid	6 May	13	8	45.1 (6.4)	93.3 *abcd* (10.4)	3.81 *cd* (1.11)
Limed pH 6.6	6 May	7+6†	24	43.5 (3.8)	128.2 (4.5)	6.25 (1.62)
Mixed pH 6.1	6 May	7+6†	24	48.3 *bcd* (3.2)	119.1 *bcd* (6.4)	5.68 (2.09)
Mixed pH 6.0	6 May	7+6†	24	49.6 *bcd* (4.9)	117.4 *bcd* (5.8)	4.57 *c* (1.73)

Note: *a*, Significantly different from corresponding value of all other groups on the same sampling date; *b*, significantly different from corresponding value of the limed group on the same sampling date; *c*, significantly different from corresponding value of the limed pH 6.6 group on the same sampling date; *d*, significantly different from corresponding value of the control group.

*N = 12, except N = 8 in the acid group.

†Days until and after change of water quality on 30 April.

Fig. 2. Accumulated mortality in the high-Al (open diamonds), moderate-Al (solid diamonds), acid (open squares), and mixed pH 5.78 (solid squares) groups of Atlantic salmon smolts after transfer to different water qualities on 9 May 1991. No mortality was recorded in the other exposure groups. See text and Table 1 for further explanation of experimental procedures and group notations.



with limed river water until 2 May. Then the water supply in two tanks was switched to either mixed acidic water as in the mixed pH.0 group (24 h mixed group) or acidic river water (24 h acid group). After 24 h and until 6 May the tanks were again supplied with limed river water (Table 2). The fish in last tank were used as controls in this short-term experiment and received limed river water for the whole expeimental period (24 h limed group).

The fish in the group referred to as the control group in Table 2 were transported to the River Vikedalselva on 22 April and then to the Ims Research Station where they were held in a 90-L outdoor plastic tank supplied with nonacidified water with a temperature of about 7°C (Table 2). Except for a longer transport time (8–9 h) (no change in water quality), these fish had experienced approximately the same handling and exposure conditions as the fish used in the exposure experiments in Vikedalselva and were thus suitable controls for fish that experienced changes in water quality.

Mortality was recorded one or two times a day. Blood samples were taken for determination of hematocrit and blood plasma Cl^- concentration, and gill tissue was sampled for determination of gill Na^+,K^+-ATPase activity (Table 3). In the short-term exposures (24 h limed, 24 h mixed, and 24 h acid groups), fish were sampled from the three tanks (N = 18, six from each) for determination of plasma Cl^- concentration before the change in water quality on 2 May, then 12 fish from each of the three tanks after 24 h in their new water qualities (unchanged quality for the controls in the 24 h limed group), and then again on 6 May (N = 12) after 3 days of recovery in limed river water (see Fig. 3).

Seawater tolerance challenge tests were started at the end of the freshwater exposures on 6 May. Before sampling fish in freshwater on that date, 25 individuals from each of the exposure groups (seven from the acid group because of the high mortality rate) were transferred to 90-L exposure plastic tanks containing 40 L of 34.5 ppt aerated seawater, which was made by adding artificial instant seawater salt to seawater of 33 ppt salinity 2 days before the start of the challenge tests. Mortality was recorded and blood samples were taken for determination of blood plasma Cl^- concentration after 24 h (N = 15 and the three survivors in the acid group) and 48 h (N = 8–10 per group). The temperature on the first day was 7–8°C but fell to about 5°C during the second night.

Water samples and analyses

Water samples were taken either daily or every second day from the outlet of the exposure tanks. The pH was measured immediately (Orion SA250 pH meter with a Radiometer GK2401C electrode). In preparation for Al analysis, samples of about 70 mL (first 50 mL for washing and subsequent 20 mL for analyses) were immediately passed through a cation-exchange column (DOWEX-50, prepared with Na^+ and analysed by the pyrocatechol violet method described by Røgeberg and Henriksen (1983)). This fraction is assumed to include primarily organic complexes of mono-

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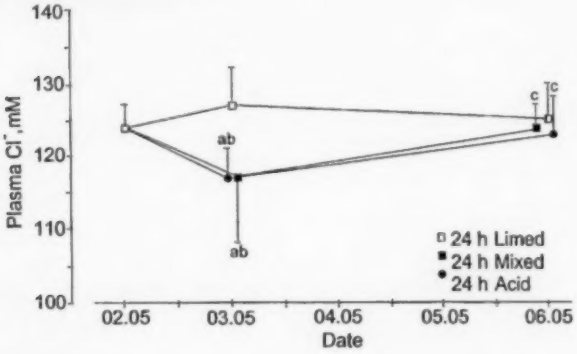
Fig. 3. Blood plasma Cl^- concentration of Atlantic salmon smolts held in limed river water sampled on 2 May (N = 18), of smolts that experienced unchanged water quality (24 h limed group, N = 12) or a decrease in water pH to 6.0 for 24 h (24 h mixed group, N = 12) or to 5.6 for 24 h (24 h acid group, N = 12) from 2 to 3 May, and of smolts from the same groups held in limed water from 3 to 6 May (N = 12). See text and Table 2 for further explantion of experimental procedures and group notations. Values are means with SD indicated (*a*, significantly different from the mean values of the fish sampled in limed river water on 2 May; *b*, significantly different from the mean values of the fish sampled in limed river water on 3 May; *c*, significantly different from the corresponding mean values of the fish sampled on 3 May).



meric Al (Al_o). The Al fraction analysed directly without any pretreatment is Al_a. The difference between these two fractions comprises the labile Al_l species that are the most toxic forms of Al (Driscoll et al. 1980). The fractionation scheme is a slight modification of that presented by Driscoll et al. (1980). All water samples were sent to the Norwegian Institute for Water Research for analysis by standard methods.

Fish sampling and analyses

Fish sampled for physiological analyses (1992 only) were killed by a blow to the head. Blood samples were collected from the caudal vessels into heparinized tubes by cutting the caudal fin. Hematocrit was determined immediately (Compur M 1100 microcentrifuge). Blood samples were centrifuged and the Cl^- concentration was assayed immediately using a Radiometer CMT chloride titrator. The gill arches of fish sampled in freshwater at the Ims Research Station on 5 May (control group) and in Vikedal on 6 May 1992 (all groups except the reference group) were dissected out, carefully dried on soft paper, and the filaments cut free. About 0.1 g of gill tissue was placed in a 1.0 mL buffer solution (Zaugg 1982) and immediately frozen in liquid nitrogen for subsequent determination of Na^+,K^+-ATPase activity. Gill Na^+,K^+-ATPase activity was assayed as described by Finstad et al. (1989) and is expressed as micrograms of inorganic phosphate per milligram of protein per hour. Gill arches were also dissected out for histological studies (A. Kvellestad, Norwegian College of Veterinary Medicine, unpublished data). One-way analysis of variance and Tukey's multiple-range test were used to test differences in values between the exposure groups. A significance level of $P < 0.05$ was chosen.

Results

Fish experiments in 1991

The fish died rapidly in the two groups exposed to water with acid and Al added and most rapidly in the high-Al group (Fig. 2). All fish were dead within 2 days. About 25% of the fish in the group exposed to naturally acidic water (mean pH 5.54) and 20% in the mixed pH 5.78 group died. No mortality was recorded in the groups exposed to water with a mean pH > 6.0.

The mortality rate in the seawater challenge tests reflected the pH of the water to which the fish were exposed prior to the tests. The highest mortality occurred in the groups that had experienced the lowest freshwater pH. All of the mixed pH 5.78 group fish tested (mean exposure pH 5.78) died, and about 90% of the two other groups exposed to mixed water (mean pH 6.06 and 6.12) died. Nearly 40% of the fish from the limed group died, whereas the mortality was lower (about 25%) and the fish died more slowly in the reference group tested.

Fish experiments in 1992

Mortality was recorded only among fish exposed to naturally acidic river water. The fish started to die on the fourth day after transfer and died rapidly the following day, until more than 60% were dead 2 days later. Thereafter, only a few fish died. The rapid increase in mortality rate coincided with a decrease in pH from 5.5 to 5.3 (Fig. 1).

After transport and 1 day in the limed river water, the Cl^- concentration was about 110 mM (Table 3). Six days later, on 29 April, the Cl^- concentration in the fish in the limed group had increased to 127 mM, which was the same level as that recorded for the control group fish sampled at the Ims Research Station on 5 May. The mean pH of the limed water during this period was 6.52 (Table 2). No similar increase was recorded in those fish simultaneously held from 23 April in water with a mean pH of 6.16 (mixed group). At the end of the experiment, only fish held at pH > 6.5 (limed pH 6.6 group) had Cl^- levels comparable with those of control fish at Ims (Table 3). All other groups showed significantly lower Cl^- levels (93–123 mM), with the lowest mean concentration in the acid group.

In the short-term exposures that started on 2 May, fish that experienced a reduction in water pH to 6.0 for 24 h (24 h mixed group) and to 5.6 for 24 h (24 h acid group) had significantly lower mean plasma Cl^- concentrations (both values 117 mM) than fish sampled in limed water (mean value 124 mM on 2 May and 127 mM on 3 May) (Fig. 3). In both the 24 h mixed and 24 h acid groups, there was a significant increase in plasma Cl^- concentrations after 3 days of recovery in limed water.

The highest mean gill Na^+,K^+-ATPase activity at the end of the experiment was recorded in the limed pH 6.6 group (Table 3). In all groups except the mixed pH 6.1 group, significantly lower mean activities were recorded. In those fish held in acid water (mean pH about 5.6) or mixed acidic and limed water of pH about 6.1 from 23 April, the mean activities were only about 60% of the activity in the limed pH 6.6 group.

Four of the seven fish from the acid group died during the first day of the seawater challenge test (Fig. 4). One of the fish from the mixed group and two from the mixed pH 6.0 group also died (25 tested in each). After 2 days the mean concentration in the limed pH 6.6 group had decreased from

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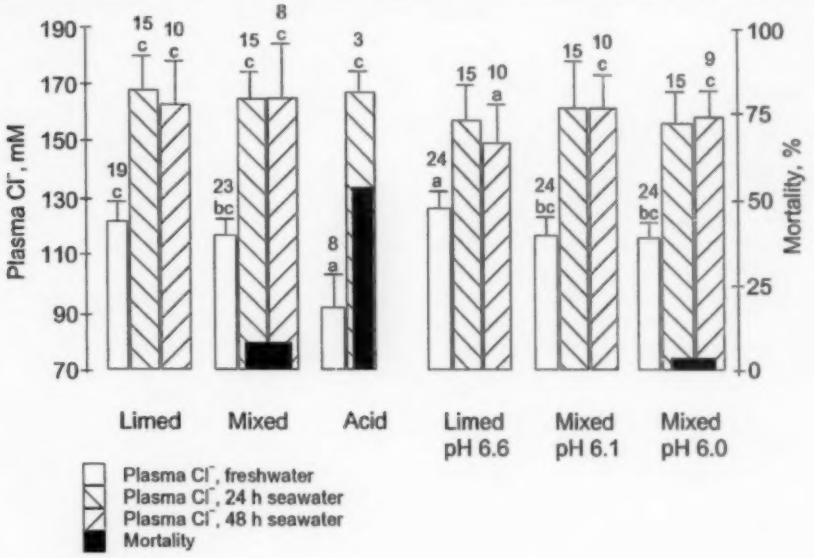
Fig. 4. Blood plasma Cl^- concentration and accumulated mortality of different groups of Atlantic salmon smolts after transfer to seawater with a salinity of 34.5 ppt on 6 May 1992, and the Cl^- concentration of the same groups in freshwater of different water qualities prior to the transfer. See text and Table 2 for further explantion of experimental procedures and group notations. Values are means with SD indicated (*a*, significantly different from all other corresponding values; *b*, significantly different from corresponding value of the limed group; *c*, significantly different from corresponding value of the limed pH 6.6 group); *N* is given above the SD.



159 to 151 mM, or 10–15 mM and significantly lower than in the other group (Fig. 4).

Discussion

Mortality and ionoregulation in freshwater

The naturally acidic water with additional Al and acid in 1991 was acutely toxic to the smolts. The pH was 5.0–5.2, and the concentration of labile Al_i was about 90 $\mu g \cdot L^{-1}$ in the group with highest mortality (LT_{50} about 10 h) and about 60 $\mu g \cdot L^{-1}$ in the other (LT_{50} about 18 h). In 1991, considerable mortality was also recorded during the 6 days of exposure, even in the water with a mean pH of 5.78 and labile $Al_i < 30$ $\mu g \cdot L^{-1}$. In 1992, there was a marked and abrupt increase in mortality during a heavy rain episode when the pH of the naturally acidic water fell from 5.5 to 5.3. These results are consistent with field observations made during acid episodes in the River Vikedalselva, where appreciable mortality of smolting Atlantic salmon has been recorded at low concentrations of labile Al_i (30–70 $\mu g \cdot L^{-1}$) (Henriksen et al. 1984; Hesthagen 1989; Hindar and Henriksen 1992).

No mortality was recorded for the smolts in water with pH > 6.0, although in the 1992 experiment, fish in the groups exposed to water with mean pH values from about 6.0 to 6.2 all suffered from sublethal ionoregulatory stress. At the end of the experiment, the mean plasma Cl^- concentrations reflected the exposure pH, with groups exposed to the lowest pH having the lowest Cl^- concentration. Only fish held in water limed to pH 6.6 had the same mean Cl^- concentration as the control group fish. Moreover the Cl^- concentration for smolts held in the limed river water (mean pH 6.52) at the start of the experiment returned to the same level as for the control group within a week. No such recovery was recorded for those that were simultaneously held in the mixed water at a mean pH of 6.16. The intial decrease in plasma Cl^- concentration was probably a combined effect of the transport and handling associated with the transfer to the exposure tanks (Staurnes et al. 1996). The short-term experiment, in which the water was changed from limed (pH 6.5) to mixed (pH 6.0) or acid (pH 5.6) for 1 day, demonstrated that even a brief exposure to a more adverse water quality can cause a moderate but significant decrease in the Cl^- concentration. However, this decrease was transient when the fish were allowed to recover for some days in limed water.

Only fish held in water with pH > 6.3 were able to maintain their osmoregulatory balance. This particular pH appeared to be the critical level with respect to the first occurrence of sublethal detrimental effects of acidic water on salmon smolts in these experiments. This assumption is supported by the fact that only the fish held in water with a mean pH of 6.57 (limed pH 6.6 group) had gills without lamellar thickening and fusion and also without histologically detectable Al on or in their gill epithelial cells (A. Kvellestad, F. Kroglund, and M. Staurnes, unpublished data).

The fact that only the fish held at pH 6.5–6.6 appeared to be unaffected does not imply that this was solely due to the lower acidity at this particular pH level. The ameliorating effects of Ca in acid water have been well documented (e.g., Rosseland et al. 1990). The higher Ca concentration experienced by fish in the limed pH 6.6 group probably contributed to the lower toxicity of the water compared with the more acidic water in which the other groups were held. The concentration of labile Al_i, however, was about the same. From a practical point of view with respect to the dosing of lime in the River Vikedalselva, it is less important whether or not the ameliorating effect was a result of lower acidity,

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higher Ca concentration, or some other factor, as long as the results indicate that the water with pH values lower than 6.5 was suboptimal for the smolts.

Gill Na^+,K^+-ATPase activity

Except for the fish held for 6 days in the mixed water at a mean pH of 6.07 (mixed pH 6.1 group), all groups had lower gill Na^+,K^+-ATPase activity at the end of the experiment than smolts held in water with a mean pH of 6.57 (limed pH 6.6 group). The fish held in either naturally acidic water or mixed water from the start of the experiment showed only about 60% of the gill activity levels of those in the pH 6.6 water. The activity levels of the fish in the natural limed river water were also significantly lower. These reduced activities may have contributed to the lower seawater tolerance and reduced freshwater plasma concentrations in these groups compared with the limed pH 6.6 group. During parr–smolt transformation, a large increase in gill Na^+,K^+-ATPase activity is of critical importance in acquiring hypoosmoregulatory ability (e.g., Zaugg and McLain 1970; Saunders and Henderson 1978; McCormick et al. 1987; Staurnes et al. 1993*a*), and the activity increase has also been suggested to be important for maintenance of the freshwater ionoregulatory homeostasis of smolts (Primmett et al. 1988; Staurnes et al. 1993*a*). The activity of the limed pH 6.6 group was four to five times the level normally recorded for parr by the method used (Staurnes et al. 1993*b*).

Exposure to Al-rich water at about pH 5 has been reported to cause a reduction in the gill Na^+,K^+-ATPase activity in both parr and smolts (Staurnes et al. 1984, 1993a, 1996; Leivestad et al. 1987; Rosseland et al. 1992), but the present experiment demonstrated impairment of enzyme activity in smolts even in water at pH > 6.0 and with a concentration of labile Al_i as low as 15–20 $\mu g \cdot L^{-1}$. Smolts are more sensitive to such reductions in enzyme activity than parr and presmolts (Staurnes et al. 1993*a*). It has been suggested that the reduction is caused by an acid/Al-induced general stress response known to impair smolting, an accelerated turnover and degeneration of the gill chloride cells in which the high Na^+,K^+-ATPase activity is localized, and (or) a direct Al inhibition of the enzyme in these cells (discussed by Staurnes et al. 1993*a*).

Sensitivity of smolts to acidic water

The results of our study confirm earlier studies that have documented the low tolerance of Atlantic salmon smolts to Al-rich acidic water (Rosseland et al. 1992; Staurnes et al. 1993*a*, 1996). However, few workers have studied the sublethal effects of exposure to water mixtures similar to those used in the present experiment. Water at pH 6.1 and about 75 µg labile $Al_i \cdot L^{-1}$ has, however, been found to be irritative to rainbow trout (*Oncorhynchus mykiss*), causing increased ventilation, coughing, and also some deposition of Al on and in the gill epithelium (e.g., Youson and Neville 1987; Neville and Campbell 1988). In our experiments with salmon smolts, distinct sublethal effects were recorded after only a day in water of even lower acidity and with mean concentrations of labile Al_i of only 15–20 $\mu g \cdot L^{-1}$. Our results also demonstrated that soft acidic water in the pH range of 5.5–5.9 might be lethal to salmon smolts even with low concentrations of Al present. Release experiments have shown that smolts released in acidic river water causing ionoregulatory failure and impairment of seawater tolerance have little chance of surviving in the sea (Staurnes et al. 1996).

The cause of the enormous increase in sensitivity to acidic water when salmon smoltify has been suggested to be mainly associated with the recruitment of more seawater-like chloride cells during smolting that strongly increase gill ion permeability (discussed by Staurnes et al. 1993*a*). This assumption is supported by the findings of McDonald et al. (1991) in that the tolerance to low pH of several fish species correlates with chloride cell density and gill ion transport activity. This indicates that sensitivity to low pH is largely a function of the intrinsic ion permeability of the gills.

Seawater tolerance

The results from the seawater challenge tests were consistent with earlier experiments that documented the negative effects of acid/Al or acid exposure on the seawater tolerance of Atlantic salmon smolts (e.g., Farmer et al. 1989; Staurnes et al. 1993*a*, 1996). In 1991, fish died in all the tested groups, with the highest mortality rate in the groups that had experienced the lowest freshwater pH. In the 1992 challenge tests, mortality was recorded in the three groups that had experienced the lowest pH, but only fish exposed to acidic river water suffered a high mortality rate. The reasons for the apparently lower seawater tolerance in the smolts in 1991 compared with those tested in 1992 are not quite clear, but the salinity was a little higher and the fish experienced more handling. The experiment also ended a week later than in the second year. The fish had a much more distinct smolt coloration, and they had very good seawater tolerance as early as mid-April. When the experiment was carried out, they were thus later in the smoltification process and possibly beginning the process of desmoltification (McCormick and Saunders 1987). Such fish have been found to be especially vulnerable to acidic water exposure and other stress factors such as handling, with respect to ionoregulation in both freshwater and seawater (Staurnes et al. 1993*b*; B.O. Rosseland, M. Staurnes, and F. Kroglund, unpublished data).

Although only fish exposed to acidic river water suffered a high mortality rate during the 1992 seawater challenge tests, those exposed to water at pH 6.0–6.3 had suboptimal hypoosomoregulatory capacity compared with the smolts held in water limed to pH 6.6 (limed pH 6.6 group). The fish in the latter group had the highest blood plasma Cl^- concentration in freshwater, the highest gill Na^+,K^+-ATPase activity, no visible structural gill changes or detectable Al deposition on or in the epithelial cells, and the highest hypoosmoregulatory capacity. Because the marine survival of Atlantic salmon smolts is correlated with their seawater tolerance at the time of release (Virtanen et al. 1991; Staurnes et al. 1993*b*, 1996), their seawater performance, if released, might have been better than that of smolts in the other groups with suboptimal hypoosmoregulatory ability. Fish with suboptimal seawater tolerance might, for example, be more vulnerable to predation. In tank experiments, osmotic stress in seawater and stress due to the presence of predators have been shown to have a synergistic effect on the mortality of Atlantic salmon smolts (Järvi 1989). Suboptimal hypoosmoregulatory ability may therefore further

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reduce smolt survival, considering the high predatory pressure encountered in estuaries (Hvidsten and Møkkelgjerd 1987; Hvidsten and Lund 1988).

Conclusions

Our experiments demonstrated the low tolerance of smolting Atlantic salmon to moderately acidic water with low concentrations of labile Al_i. Compared with the smolts held at pH > 6.5, those held at mean pH < 6.2–6.3 and Al_i as low as 15–20 $\mu g \cdot L^{-1}$ had suboptimal smolt quality. Even a short-term exposure of 1 day at pH 6.0 affected iono-regulation. These results support the hypothesis that Atlantic salmon stocks may be affected by acidification even in rivers that are not chronically acidic and not normally regarded as being in danger of acidification because of the very low tolerance occurring at the smolt stage (Staurnes et al. 1993*a*).

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Survival of pink salmon (*Oncorhynchus gorbuscha*) fry to adulthood following a 10-day exposure to the aromatic hydrocarbon water-soluble fraction of crude oil and release to the Pacific Ocean

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Abstract: Saltwater-acclimated, coded-wire tagged, and adipose fin clipped pink salmon (*Oncorhynchus gorbuscha*) fry were exposed for 10 days to seawater (control) or 25–54 $\mu g \cdot L^{-1}$ (low dose) or 178–349 $\mu g \cdot L^{-1}$ (high dose) of the water-soluble fraction (WSF) of North Slope crude oil. The WSF was composed primarily of monoaromatics and was acutely lethal to the fry: 96-h LC_{50} ranged from 1 to 2.8 $mg \cdot L^{-1}$. After exposure the fry (30 000 per treatment) were released into the Pacific Ocean to complete their life cycle. The experiment was replicated in 1990, 1991, and 1992. There was no consistent significant dose-dependent effect of the 10-day exposure to the crude oil WSF on growth of the pink salmon prior to their release. Adult pink salmon from this experiment were captured in fisheries and also recovered from their natal Quinsam River, British Columbia. Pink salmon from each treatment group were recovered in similar numbers. Exposure of populations of fry to the WSF of crude oil and release to the Pacfic Ocean did not result in a detectable effect on their survival to maturity. Fry from all treatment groups incurred typically high mortality following release, and there were no discernible effects on survival that were attributable to exposure to the WSF of crude oil.

Résumé : Des alevins de saumon rose (*Oncorhynchus gorbuscha*) à nageoire adipeuse coupée, porteurs d'une micro-marque codée et acclimatés à l'eau salée, ont été exposés pendant 10 jours à de l'eau de mer (témoin) ou à des concentrations de 25 à 54 $\mu g \cdot L^{-1}$ (concentration faible) ou de 178 à 349 $\mu g \cdot L^{-1}$ (concentration élevée) de la fraction hydrosoluble du pétrole brut du versant Nord. Cette fraction hydrosoluble comprenait principalement des composés monoaromatiques à létalité aiguë pour les alevins : la CL_{50} à 96 h s'établissait entre 1 et 2,8 $mg \cdot L^{-1}$. Après avoir été exposés à ces produits, les alevins (30 000 par expérience) ont été libérés dans l'océan Pacifique pour terminer leur cycle vital. L'expérience a été répétée en 1990, 1991 et 1992. L'exposition de 10 jours à la fraction hydrosoluble du pétrole brut n'a eu aucun effet constant important lié à la dose sur la croissance du saumon rose avant sa libération dans l'océan. Les saumons roses adultes utilisés pour cette expérience ont été capturés dans les pêches et récupérés aussi dans la Quinsam, leur rivière natale en Colombie-Britannique. On a récupéré le même nombre de saumons roses appartenant à chacun des groupes de l'expérience. L'exposition de populations d'alevins à la fraction hydrosoluble du pétrole brut et leur libération dans l'océan Pacifique n'ont eu aucun effet décelable sur leur survie jusqu'à la maturité. En général, on a enregistré une mortalité élevée chez les alevins de tous les groupes après leur libération, et aucun effet discernable sur la survie n'était attribuable à l'exposition à la fraction hydrosoluble du pétrole brut.

[Traduit par la Rédaction]

Introduction

Prior to the *Exxon Valdez* accident in 1989, which brought renewed attention to the potential effects of spilled oil (Rice et al. 1996), the possibility of future oil and gas exploration and production (Chevron Canada Resources Ltd. 1982) and the presence of oil tanker traffic along the British Columbian coast had prompted several government initiatives to exam-

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ine the potential consequences of oil spillage to Pacific marine ecosystems (FEARO 1986; States/British Columbia Oil Spill Task Force 1990). The research project reported herein was designed to address some of those concerns in relation to the potential effects of hydrocarbons on salmonids.

The spillage of about 42×10^6 L of crude oil from the *Exxon Valdez* supertanker resulted in research that has increased our knowledge of the effects of oil on avian and aquatic biota (refer to Wells et al. 1995; Rice et al. 1996) and also confirmed how difficult it is to assess the effects of spilled oil under field conditions. For example, assessments of the effects of spilled oil on migratory fishery resources are frequently hindered by a lack of knowledge of the duration of exposure to the specific and changing environmental conditions. Accordingly, reliance is often placed upon strong and compelling inferences from valid scientific investigations to ascertain impacts (Rice et al. 1996). However, the assignment of significance to such impacts has led to controversy among scientists from the oil industry and governments regarding the effects of oil that was spilled from the *Exxon Valdez* oil tanker on the aquatic organisms of Prince William Sound (Brannon and Maki 1996; Rice et al. 1996). That spilled oil had the potential to harm the pink salmon (*Oncorhynchus gorbuscha*) of this region was not disputed; rather, it was the precise determination of effects on populations that was in question. Most recently, Bue et al. (1998) suggested that chronic damage occurred to some populations of pink salmon exposed to oil spilled from the *Exxon Valdez* and provided evidence that increased mortality of pink salmon embryos was linked to the previous exposure of parental stock to oil.

Laboratory experiments that determined the effects of crude oil and its constituents on pink salmon (Rice 1973; Rice et al. 1977; Korn et al. 1979; Moles and Rice 1983; Babcock 1985; Moles et al. 1987; Wang et al. 1993) eliminated many of the potential problems associated with determining such effects under field conditions. These studies, typically of short duration, indicated the potential for harm to pink salmon through exposure to oil, which, by inference, implied that there would also be the potential for harm under field conditions. The limitations of laboratory-based studies, to mimic conditions found in the wild and to define impacts of spilled oil in the field, prompted us to consider an experiment that provided a greater relevance to the assessment of effects on populations of salmonids than that which was available through standard laboratory toxicity testing. Longer term exposures of fish to contaminants have the potential to provide highly relevant information, and while these chronic effects studies have been undertaken on certain fish species using life cycle tests, such an approach for salmonids has generally been considered to be financially and logistically impractical (Environment Canada 1998). However, the availability of pink salmon from a coastal hatchery, and an existing federal government program that recovered and identified marked adults, facilitated the undertaking of such a study.

Our primary objective was to determine whether exposure of saltwater-acclimated pink salmon fry to sublethal concentrations of the water-soluble fraction (WSF) of crude oil had a significant effect on their subsequent ocean survival to adulthood. Pink salmon have a comparatively short life span relative to other salmonid species, and this permitted three experimental releases and the return of adults within 4 years. Also, in the early part of their life, pink salmon are biased towards the surface of the water and frequently occupy shallow nearshore habitats (Heard 1991), which places them at particular risk in the event of oil spillage (Geiger et al. 1996; Willette 1996). Newly emerged saltwater-acclimated fry are also particularly sensitive to dissolved hydrocarbons (Moles et al. 1979, 1987), and their small size at emergence was conducive to the handling and exposing of high numbers to the WSF. We used the WSF of crude oil because of the previously documented lethal and sublethal effects of these aromatic hydrocarbons to these fish and the potential for such compounds to be elevated in surface waters at the time of oil spillage. The rapid dissipation of these toxic aromatic hydrocarbons has been documented for a number of crude oil spill events; however, in the event of an oil well blowout or the spillage of refined products, the potential for these hydrocarbon fractions to remain elevated is increased. Even after the spill of crude oil from the *Exxon Valdez* in 1989 under calm sea conditions, determinations of hydrocarbons in the water column were not made until days afterwards (Neff and Stubblefield 1995; Short and Harris 1996), and polycyclic aromatic hydrocarbons (PAHs) contaminated seawater to a depth of at least 5 m and were at concentrations up to 6 $\mu g \cdot L^{-1}$ 1–2 weeks after the spill. Little information was gathered on the concentrations of monoaromatic hydrocarbons, and the potential effects on aquatic resources were generally assessed in relation to the more persistent PAHs (Rice et al. 1996; Bue et al. 1998). The aromatic hydrocarbon concentrations used in our study were high relative to the concentrations of these hydrocarbons determined days after spill events, but potentially represented levels that could occur immediately upon oil spillage (i.e., before the rapid volatilization of these low molecular weight compounds, which make up over 95% of the WSF).

To our knowledge, this study, which took 6 years to complete, is the first replicated life cycle study that has examined the effects of the WSF of crude oil on salmon.

Materials and methods

Study location

The exposure of juvenile pink salmon to the aromatic hydrocarbon WSF of crude oil was undertaken near the town of Campbell River, Vancouver Island, in the northwestern portion of the Strait of Georgia, British Columbia, Canada (Fig. 1). The adjacent marine waters of Discovery Passage are contiguous with those of Johnstone Strait, which enters the Pacific Ocean. The test fish came from the economically important Quinsam River stock, Campbell River, which supports an annual harvest of 80 000 – 500 000 adults (I. Birtwell, Department of Fisheries and Oceans, West Vancouver Laboratory, West Vancouver, B.C., unpublished data). A temporary field laboratory and saltwater rearing facility was constructed near the mouth of the Campbell River. The proximity of this facility to the hatchery facilitated the rapid transport of the fry, which was accomplished within 20 min.

Experimental system

The seawater experimental system outlined in Fig. 2 comprised a pumping system, a seawater air-equilibration tower and distribution system, three fish holding troughs, a dissolved hydrocarbon

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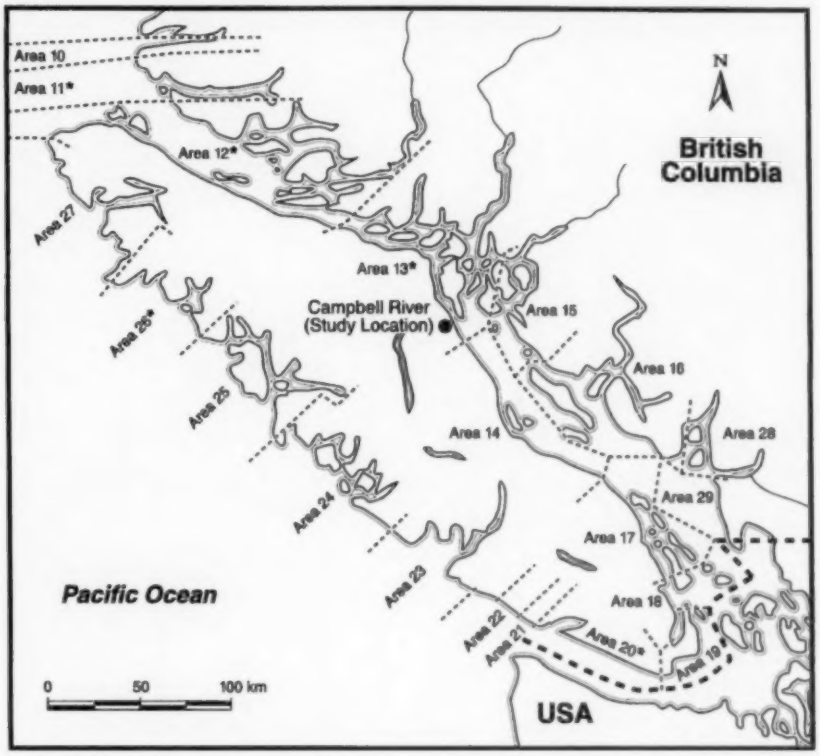
Fig. 1. Location of the field laboratory site and the source of test pink salmon at Campbell River, Vancouver Island, B.C. Department of Fisheries and Oceans fisheries catch statistical areas are depicted along the coastline for reference to the capture locations (asterisks) of adult experimental fish.



preparation system, and a flow-through toxicant exposure apparatus. Seawater was pumped from a submerged, screened intake located 2.5 m above the sea floor. A 0.25-hp Franklin single-stage stainless steel well pump in series with a 0.5-hp nylon and stainless steel pump delivered seawater at 225 L·min^{-1} (at high tide) to a 2 m × 15 cm PVC air-equilibration column. Air-equilibrated seawater (9.8–10.4 mg dissolved oxygen·L^{-1}, salinity 28–30‰) was fed by gravity from a 200-L polyethylene reservoir to the fish holding troughs, dissolved hydrocarbon preparation system, and the flow-through toxicant exposure apparatus.

Each of three 2-m^3 holding troughs received 25 L seawater·min^{-1}. Black nylon mesh covers prevented fish escape, reduced direct sunlight, and provided shade. A water depth of 60 cm was maintained in the troughs. The 90% water replacement time for these troughs was 4 h. The holding troughs, bioassay apparatus, and hydrocarbon preparation system were housed in a temporary wooden structure glazed with 6-mm UV-grade polyethylene film.

Fry treatment

Individual emergent pink salmon fry were identifiable through the insertion of half-length coded-wire tags (CWTs). At the Quinsam Hatchery, the pink salmon fry were anaesthetized in 0.1% 2-phenoxyethanol, marked by adipose fin removal (using scissors), and then tagged with half-length binary-coded-wire tags using a Mark IV tagging machine (Northwest Marine Technology Inc., Shaw Island, Wash.). Tagged fry were assigned to one of three experimental groups. A 24- to 48-h evaluation of tag loss was made by passing 150 fish through a Northwest Marine Technology Inc. quality control device. Overall tag retention at release (27–29 days) was determined for each treatment group.

Fry were transported from the hatchery to the temporary laboratory experimental facility in continuously aerated freshwater in 60-L polyethylene containers at a loading density of 10–20 g·L^{-1}. At the field laboratory, seawater (28–30‰) was siphoned at 6 L·min^{-1} into each container, thereby replacing the freshwater in 15–30 min, after which the fry were poured directly into the appropriate holding trough.

Fry were given a food ration of 2.5–3% of their body weight per day, an amount sufficient to produce moderate growth while preserving acceptable dissolved oxygen levels within the holding troughs. New batches of fish were introduced into the troughs after the final evening feeding and were not fed until the following morning. Fry were fed frozen Biodiet Starter Mash No. 1 followed by Biodiet Starter Mash No. 2 (Bioproducts Inc., Warrenton, Oreg.). Seawater delivered to each of the holding troughs was coarse-screened through multiple layers of 1-cm nylon net; therefore, a small and equal amount of natural food was always available. The sides and bottoms of each trough were siphoned after the last feeding each day to remove uneaten food and feces. Any mortalities were recorded and the carcasses removed twice daily. Fish loading densities relative to inflow waters increased from 0.25 kg·L^{-1}·min^{-1} at the end of the tagging period to 0.42 kg·L^{-1}·min^{-1} at the time of release, due to the growth of the fish during the experimental period.

Dissolved oxygen was measured twice daily (±0.1 mg·L^{-1}) with a portable YSI model 57 (Yellow Springs Instruments Ltd., Yellow

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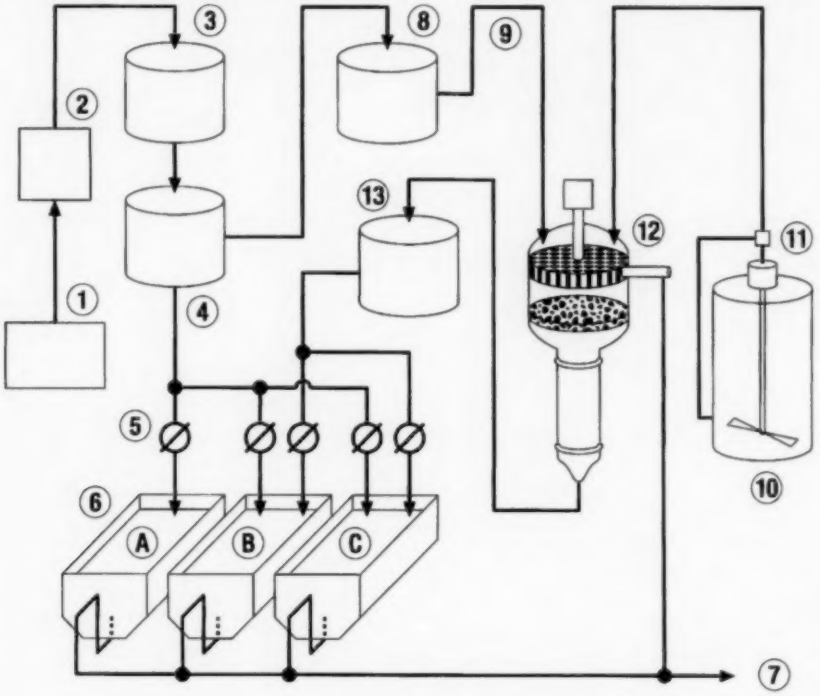
Fig. 2. Main components of the apparatus used to expose juvenile pink salmon to the WSF of crude oil: 1, submerged seawater supply pump; 2, secondary onshore seawater supply pump; 3, air-equilibration column; 4, seawater header tank; 5, flow control valves; 6, fish holding and exposure troughs (A, control; B, high WSF; B, low WSF); 7, drain to waste sump and oil recovery; 8, secondary filtered-seawater reservoir; 9, seawater supply to WSF generation apparatus; 10, crude oil reservoir; 11, pump for oil delivery to WSF generation apparatus; 12, WSF generation apparatus (after Moles et al. 1985); 13, WSF reservoir.



Springs, Ohio) calibrated against air-saturated seawater, while temperature and salinity were measured (±0.1°C and ±0.5‰) with a custom-made recording oceanographic probe (Axys Environmental Consulting Ltd., Sydney, B.C.).

Crude oil WSF

The WSF of North Slope crude oil was produced with a glass extraction column similar to that used by Moles et al. (1985) but modified for a higher production volume. The critical element of this extraction column was a pipette plate situated above a constantly replenished floating oil layer, which dispersed a continual "rain" of small seawater droplets onto the oil. The higher specific gravity seawater droplets sank through the oil layer, dissolving low molecular weight aromatic hydrocarbons in the process. Subsequently, these hydrocarbon-carrying droplets exited the oil–water interface and coalesced to form a particulate-free WSF that flowed by gravity through a glass column and tubing to a reservoir. A slowly rotating (60 rpm) stirrer positioned in the column assisted in stabilizing the oil–water interface and thereby prevented the entrainment of whole oil into the WSF stream. Fresh crude oil was pumped to the WSF extraction column oil layer at the rate of 4–6 mL·min^{-1}. Spent crude oil, from which water-soluble components had been extracted, was automatically siphoned from the bottom of this layer to a waste storage container for subsequent disposal. The flow of seawater through the extraction column was 2.2 L·min^{-1}. Gravity siphons of clear 10 mm inside diameter PVC tubing distributed WSF from the reservoir to the holding troughs.

In 1991 and 1992, water samples for dissolved hydrocarbon analyses were collected twice daily at middepth in each trough in 40-mL screwcap Teflon-sealed glass vials and preserved for shipment with a single crystal of mercuric chloride. Labelled vials were sealed with black vinyl tape and transported by air in insulated containers (4 ± 2°C) to AGAT Laboratories (Calgary, Alta.). Analyses were initiated less than 48 h after collection using a modified EPA 602 GC-MS method for purgeable aromatic hydrocarbons (U.S. Environmental Protection Agency 1984). Analytical problems in 1990 did not permit characterization of the WSF produced in the extraction system during experiments. Accordingly, retrospective estimates of aromatic hydrocarbon concentrations used in the 1990 experiment were made at the conclusion of the exposure when the WSF production was evaluated.

Acute toxicity

The acute toxicity of the crude oil WSF to juvenile pink salmon was determined over 96 h using a flow-through technique prior to the 10-day aromatic hydrocarbon exposure. One bioassy experiment, which used a range of concentrations of the WSF, was carried out in each of the three years that the pink salmon were exposed and released. The results of the first bioassay were used to determine the approximate range of the sublethal concentrations of the crude oil WSF to be employed in the 10-day exposure and in subsequent years as a check on the relative toxicity of this oil fraction. Four-litre glass jars were the test aquaria, and PVC control valves provided proportional mixing of seawater and WSF flows. Each treatment jar received air-saturated seawater at 0.5 L·min^{-1} containing a specific concentration of the WSF. Determinations of the hydrocarbon concentration in the dilutions of the WSF were performed by AGAT Laboratories. Eighty fish were randomly se-

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lected from the three holding troughs and equally distributed among eight 4-L glass jars, resulting in a loading density of 0.55 g·L^{-1} (10 fish per container). The 90% replacement time for water in the aquaria was 0.4 h. Fry were not fed during the bioassays.

Exposure to crude oil WSF

A 10-day hydrocarbon exposure period was chosen after consultation with the oil and gas industry regarding the average duration of oil spill events from exploration and production facilities.

The aromatic hydrocarbon concentrations were selected to be in the sublethal range for juvenile pink salmon based on previous toxicological work by Moles et al. (1987) and ourselves. The concentrations of hydrocarbons were within the range anticipated immediately after spill events and oil well blowouts; except for confined and very turbulent waters, hydrocarbon concentrations rarely approach 1 mg·L^{-1} and are more often in the parts per billion range (e.g., Calder and Boehm 1981; Maki 1991; Short and Harris 1996).

Two groups of pink salmon fry received either a high dose (178–349 µg·L^{-1}) or a low dose (25–54 µg·L^{-1}) of the aromatic hydrocarbons. An equivalent group of tagged fry were maintained in seawater as controls, and all fry were exposed to the experimental conditions for 248–250 h. Levels of dissolved aromatic hydrocarbons determined in several control water samples (<6 µg·L^{-1}) may have represented sampling or analytical error (practical quantification limit was 0.1 µg·L^{-1}) or perhaps low background hydrocarbon levels from nearby marine industrial and recreational centers.

Growth during hydrocarbon exposure

Before the release of the fry in 1991 and in 1992, random samples of fish were removed from the holding troughs until about 300 fish from each treatment group were obtained. Each sample was maintained in flowing seawater in 60-L plastic containers for 8–12 h before being euthanized with tricane methanesulfonate (MS 222) anaesthetic and then weighed (± 0.01 g), and its standard length (nose to hypural plate, ±1 mm) was measured. During 1992, a further 300 fry per treatment group were removed just before hydrocarbon exposure to provide a pretreatment sample (pretreatment length and weight in 1990 and 1991 were obtained during the tagging program, thus encompassing the tagging and holding periods).

Fry release

Following the 10-day exposure to the aromatic hydrocarbons, the control and treatment populations were released into the marine waters of Discovery Passage to complete the natural saltwater phase of their life in the Pacific Ocean. An evening release avoided the potential for excessive avian predation. A 30-m flexible, smooth-walled, 5-cm-diameter PVC hose was connected to the outlet standpipe of each holding trough and the other end coupled to a solid PVC pipe extension positioned 2–3 m beyond the tide line in 40- to 60-cm-deep water. The fry were transferred into the marine waters of Discovery Passage by draining the holding troughs through the hose (transit time 10–12 s).

Adult returns

From August through October in 1991, 1992, and 1993, adult pink salmon (without adipose fins) returning to their natal Quinsam River were collected during postspawning "dead pitch" surveys and counting fence checks on the river, and from broodstock holding at the adjacent hatchery. An identification number, sex, length (postorbital to hypural), and, for some fish, weight (± 0.1 kg) were recorded. Heads were removed, frozen, and labelled for CWT detection and recovery. Adipose fin clipped pink salmon were also recovered from the commercial fishery. Length (postorbital to hypural), location, and date of capture were recorded for these fish, and the heads were labelled and retained for CWT recovery and identification. A small number of CWTs were lost during the dissection and analysis, or were unreadable. Adipose fin clipped adult fish captured in the commercial fishery or returning to spawn were assumed to be part of this investigation, since, with the exception of fish from the Quinsam River marking program (adipose fin and ventral fin removed), no other group of similarly marked pink salmon was reported from the study area (B. Anderson, Department of Fisheries and Oceans, Vancouver, B.C., personal communication).

Results

We calculated that 86% (230 355) of the 268 680 pink fry tagged during the investigation had CWTs at the time of release. The group that received the high WSF dose had the greatest number released (77 463) and the low-dose group the least (75 635). Tag retention values from this investigation compare favorably with those reported by Kaill et al. (1990) and Peltz and Miller (1990) in large-scale (about 100 000 pink salmon fry per year) tagging operations in Alaska. Detailed information on tagging methodology and results has been reported by Fink et al. (1995).

During the saltwater holding and treatment period, mean afternoon water temperatures ranged from 8.6°C (1992) to 9.7°C (1990). Average salinity ranged from 28.4‰ (1992) to 29.7‰ (1990). Within-year variation in salinity was less than 1.5% over each experimental period and did not vary significantly between individual exposure troughs. Average dissolved oxygen concentrations in the exposure troughs were highest in 1992 (8.3–8.5 mg·L^{-1}) and lowest in 1990 (7.2–7.4 mg·L^{-1}). The difference reflects the generally warmer climate and seawater in 1990 and that part of the 1990 holding period before the installation of the seawater air-equilibration column. Despite using air-equilibrated seawater at a flow of 25 L·min^{-1}, which gave a 90% trough replacement time of 4 h, the dissolved oxygen concentrations decreased in response to the growth of the fish and the corresponding increase in loading density and oxygen requirements. Towards the end of the 10-day exposure, values for dissolved oxygen decreased to 72–75% (1990), 68–77% (1991), and 76–83% (1992) of air-saturated levels.

Aromatic hydrocarbon WSF

Dissolved aromatic hydrocarbon concentrations in the holding troughs varied between years in response to changes in incoming seawater and air temperatures, consequent differences in extraction efficiency of the WSF production column, as well as minor differences in composition of the whole crude oil. The WSF consisted of benzene (43%), toluene (38%), *o*-, *m*-, and *p*-xylenes (15%), ethylbenzene (3%), and naphthalene (1%). The composition of North Slope WSF is very similar to that of Cook Inlet (Korn et al. 1979) and Prudhoe Bay crude oil (Moles et al. 1985). The nominal concentrations of WSF, 500 µg·L^{-1} for the high-dose and 50 µg·L^{-1} for the low-dose treatment groups, were chosen for the exposure; however, hydrocarbon losses within the preparation and delivery systems, as well as evaporative losses from the exposure troughs, reduced the aromatic hydrocarbon concentrations to which the fish were exposed. High-dose WSF concentrations varied among years from 178.3 ± 78.4 µg·L^{-1} (1992, n = 20) to 348.6 ± 58.4 µg·L^{-1}

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Table 1. Capture of adult pink salmon with legible CWTs from the coastal fisheries and a natal stream and the Quinsam River Salmon Hatchery.

	Department of Fisheries and Oceans Catch Statistical Area						
Year	11	12	12 or 13	13	20	26	Quinsam River and Hatchery
1991	—	26	3	14	1	—	169
1992	1	14	19	64	—	11	317
1993	—	2	—	77	—	—	172

(1991, n = 41) and the low-dose concentrations from 25.3 ± 6.8 $\mu g \cdot L^{-1}$ (1992, n = 20) to 54.2 ± 17.5 $\mu g \cdot L^{-1}$ (1991, n = 40). Variation in aromatic hydrocarbon concentrations was greater than anticipated and it was not possible to compensate for changes in production of the WSF by manipulating seawater flow rates. The seawater flow rate (25 $L \cdot min^{-1}$) that was required to maintain acceptable dissolved oxygen concentrations in the exposure troughs also required the WSF extraction column to be operated at full capacity. Higher flow rates in the extraction column produced unacceptable instability at the oil–seawater interface and subsequent intrusion of oil droplets into the WSF.

Acute toxicity of crude oil WSF

The toxicity of the WSF to pink salmon fry was similar among years, and the 96-h LC_{50} values were 2.2, 2.8, and 1.0 $mg \cdot L^{-1}$ for 1990, 1991, and 1992, respectively. By comparison, Korn et al. (1979) reported 96-h median tolerance limits of 1.45–1.77 $mg \cdot L^{-1}$ for Cook Inlet crude oil, and Moles and Rice (1983) reported the same concentration (1.2 $mg \cdot L^{-1}$) of crude oil or naphthalene as the 96-h LC_{50} and 40-day LC_{50} value for pink salmon juveniles.

Feeding and growth during hydrocarbon exposure

Feeding intensity was vigorous in each treatment group. All fry were fed an unsatiating ration of 2.5–3.0% of body weight per day.

Although there were significant differences in the growth of the pink salmon juveniles between treatment groups over the 10-day exposure period, these differences were not consistent between years or between treatment groups. Thus, we deduced that the 10-day exposure of pink salmon fry to dissolved aromatic hydrocarbons did not result in consistent significant dose-dependent differences in growth among treatment groups. However, fry from each treatment group grew during the hydrocarbon exposure period, and standard length (all treatment groups combined) increased from 0.16 to 0.24 $mm \cdot day^{-1}$ and mean weight increased between 3 and 6 $mg \cdot day^{-1}$. Mean lengths for all treatment groups after hydrocarbon exposure ranged from 33 to 35 mm. Mean weight ranged from 0.29 to 0.43 g, and mean condition factors were between 0.81 and 1.00.

Survival in holding troughs

Few deaths occurred in the troughs each day and mortality was not significantly affected by hydrocarbon exposure. Differences in cumulative mortality (total mortality of fry in each trough) were significant with respect to study year, with 1991 fry mortality (0.07%·day^{-1}) less than 50% of that in 1990 or 1992. Although we considered normalizing the 1991 data to correct for this lower overall mortality before testing for a relationship between hydrocarbon exposure and fry mortality, the genetic separation of odd- and even-year pink salmon, together with the significantly smaller size of emerging fry in 1991, mitigated against such action.

The daily mortality for all treatment groups combined ranged from 0.04 to 0.08% between 1990 and 1993. Daily mortality increased over the last 5 days of the exposure period and ranged between 0.03 and 0.19%.

Fry release

The release operation proceeded efficiently in 1990 and 1991, but in 1992, a rapidly falling tide, minimal wave activity, and the possible attraction to artificial light in the release area resulted in the shoreline stranding of an estimated 1000 fry (1.1%) from a release of 88 331. Sixty-nine dead fry were collected from the adjacent beach 8 h after the release. Of these fish, 59.4% were controls and 15.9% were from the high-dose and 8.7% from the low-dose treatment groups. Tags were not recovered from the remaining 16%. This trend in mortality followed the release strategy in which the control group had been released first, followed by the high- and low-dose groups.

Adult recoveries

Adult pink salmon recovered from the commercial fishery were predominantly from Johnstone Strait (Department of Fisheries and Oceans catch statistical areas 12 and 13) near their natal Quinsam River (Table 1); only 6% were caught in other areas. The number of adult pink salmon recovered varied between years, with 46, 243, and 517 obtained in 1991, 1992, and 1993, respectively. The number of adult fish with legible CWTs was 44 (95.8%), 109 (44.9%), and 80 (15.5%) for the same years. In relation to the total estimated number of tagged fry released each year, this represented a recovery of 0.06% (1991), 0.13% (1992), and 0.11% (1993). When data from all years were combined, 81 control fish, 79 low-dose fish, and 73 high-dose fish were obtained from the commercial fishery (Table 2). This implied that the within-year variation in survivorship between treatment groups was considerable. For example, in 1991, twice as many high-dose adults (20) returned as did control adults (9), whereas in 1993 the opposite occurred, with 32 control fish and only 16 high-dose fish returning.

From 1991 to 1993, a total of 658 adult pink salmon with legible CWTs were obtained from those that returned to the Quinsam River. This total included fish from the counting fence and holding pens and from postspawning dead pitch surveys (Table 1). Of these, 220 were from the high-dose and 216 from the low-dose treatment groups and 222 were from the control groups (Table 2).

Tagging required up to 14 days to complete and resulted in test populations comprising individuals of different age and background. Within each treatment group, fry that were tagged early in the program had codes different from those tagged later, thus permitting an assessment as to whether those tagged early in the program survived to adulthood in higher numbers than those tagged later (Fink et al. 1995). A comparison was made between the expected number of recoveries of fish in each tag code category and the actual proportion recovered. More fry (18.1%) tagged early in the

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Table 2. Number of adult pink salmon with legible CWTs that were recovered between 1991 and 1993 from the coastal fisheries and their natal stream and the Quinsam River Salmon Hatchery relative to a 10-day exposure as fry to the WSF of crude oil.

	Fry treatment group		
Capture location	Control	Low WSF dose	High WSF dose
Fisheries	81	79	73
Quinsam River and Quinsam River Salmon Hatchery	222	216	220
Total recovery	303	295	293
Percentage of released population with tags	0.39	0.39	0.38

1990 high-dose group were recovered as adults than would have been predicted based on the proportion of these fish in the group that was released. Similarly, low-dose (14.6%) and control fry (8%) tagged early in 1990 were also recovered as adults in higher proportions when compared with the later-tagged fry. However, the differences were only significant for the high- and low-dose fry (χ^2 goodness-of-fit, $p \leq 0.05$). This incidence of higher survival to adult of fry tagged early in the 1990 tagging program was not repeated in 1991 and 1992, suggesting that some factor unique to that year was responsible for the differences. The total number of adult fish obtained with legible tags from the commercial fishery and the natal stream was 891, of which 34.0% were from the control group, 32.9% from the high-dose group, and 33.1% from the low-dose group. The release of 230 355 tagged pink salmon fry over 3 years resulted in a recovery of 303, 295, and 293 adults with legible tags from the control and low and high hydrocarbon exposure groups, respectively. The corresponding recoveries were 0.39, 0.39, and 0.38% (Table 2).

A difference in tag retention between the fish taken from the commercial catch and those from the natal Quinsam River suggested that some adipose fin clipped fish were present in the salmon population that did not originate from our study (Fink et al. 1995). Using the tag retention information derived from adult pink salmon that entered the Quinsam River, a correction factor was applied to provide a more accurate estimate of the recovery of adults from this study. Based on this information, 0.4, 0.7, and 0.6% of the fry with tags were recovered in 1991, 1992, and 1993, respectively, an overall recovery of 0.6% (1324 individuals) from 230 355 tagged fry. This is not a survival estimate for the experimental populations, as we do not have data on the total number of pink salmon captured in all fisheries or information on the sampling efficiency on the Quinsam River.

Adult sex, length, and weight

Data on the sex, length, and weight of adult pink salmon taken from the Quinsam River have been reported by Fink et al. (1995). Control males were more numerous than females (122 versus 100) when all data were combined. Low-dose males returned in numbers comparable with those of low-dose females (106 versus 110), while high-dose males were less numerous than high-dose females (105 versus 115). The greatest departure from these relatively close sex ratios was in the 1993 high-dose fish, when females outnumbered males by a factor of 2.6:1 (36 versus 14).

While differences in adult weight as a function of treatment group were minor, differences between experimental years were considerable. Male and female adults were significantly shorter and lighter in 1992 than in either 1991 or 1993, possibly reflecting genetic differences between odd- and even-year stocks (Groot and Margolis 1991). On average, control males weighed less (1.18 ± 0.31 kg, n = 122) than either low-dose (1.26 ± 0.32 kg, n = 104) or high-dose (1.23 ± 0.30 kg, n = 105) males; however, the differences were not significant. Low-dose females in 1992 were significantly heavier (1.11 ± 0.17 kg, n = 55) than high-dose females (1.02 ± 0.10 kg, n = 43). They were also slightly heavier than high-dose females in 1991 and 1993, but the differences were not significant. During the same period, low-dose females tended to be equal to, or heavier than, the control females, but none of the differences was significant.

Two trends in the adult length and weight data were found: control males tended to be lighter on average than low-dose males, while low-dose females were, on average, heavier than high-dose females and heavier than or equal to control females. Neither trend was significant ($p \leq 0.05$). No significant difference in mean length of commercially caught fish as a function of treatment was observed ($p \leq 0.05$, postoccipital to hypural plate range 468–486 mm).

Discussion

We expected that the number of recovered adult pink salmon would differ among the treatment groups and thereby reveal whether sublethal exposures of fry to the aromatic hydrocarbon WSF of crude oil had resulted in differential survival under natural conditions. We concluded, however, that the exposure of populations of pink salmon fry to 25–54 or 178–349 $\mu g \cdot L^{-1}$ of the aromatic hydrocarbon WSF of North Slope crude oil for 10 days did not result in a significant difference in survival to adulthood relative to control fish. While between-year variation in the number of recovered adults was considerable, the combined data for the three replicate experiments revealed that adults from the control group were only 1.1% more numerous than those from the high-dose group, while the numbers from the low-dose group were between these two groups. All the fish returned in a state of advanced reproductive maturity.

In this unique experiment, we chose to evaluate the effects of the hydrocarbon treatment under "natural" conditions, and accordingly, we sacrificed a measure of control by releasing the fry to complete the saltwater phase of their life in the Pacific Ocean. Thus, we gained a more relevant and ecologically meaningful result than that which would have been obtained had the fish been held after treatment under artificial conditions until maturity. However, the necessity of rep-

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licating the experiment three times in succession (a consequence of logistical constraints) introduced the potential for between-year variability in experimental and oceanic conditions, which may have masked potential differences in survival among treatment groups. Studies reviewed by Pearcy (1992) indicate extreme differences in ocean survival of pink salmon from fry to adult. Where natural variation is high, long-term effects resulting from brief toxic exposures are difficult to elucidate. Barnthouse et al. (1989) pointed out that "for typical fisheries data sets, where sample size is limited and variability is high, changes of 50% or more may be undetectable using conventional statistical criteria." Thus, even if there was a different survival rate between treatment groups and among years, it may have been obscured by such variability. That aside, the high mortality in all the populations that we released would have mitigated against determining any subtle effects of exposure to the crude oil WSF on survival to adult.

The nighttime release that we chose to simulate natural downstream migration (to avoid the potential immediate daytime predation of the fry by large flocks of seabirds present near the study site) may have eliminated a potential source of predation that could have contributed to differential mortality among treatment groups. In addition, the effect on fry survival to adulthood of a sublethal toxic challenge administered within a protected, food-enhanced environment for a 10-day period may be overshadowed by the influence of later, more rigorous natural and anthropogenic challenges. Over the course of the investigation, at least 230 000 tagged fry were released from the experimental holding troughs, and only 891 (0.4% with legible tag codes) were recovered. Although the fate of 99% of the fry used in this experiment remains unknown, we are confident that the vast majority of test subjects were neither recaptured (and identified) in the commercial fishery nor returned as mature adults to the Quinsam River (nor were any experimental fish observed in other nearby river systems). There is a possibility that postexperimental selection for, or against, oil-exposed fish could have taken place but remained undetected because of, in this instance, the more significant oceanic mortality events (predation, disease, fishing).

Furthermore, the experiment that we undertook did not simulate all the conditions that may be encountered by juvenile pink salmon at the time of a spill of crude oil in the wild, and the results obtained must be viewed accordingly. For example, the absence of oil droplets or floating oil, absence of oiled prey, and absence of natural predators were potentially significant issues that were not addressed. Schwartz (1984) demonstrated that the feeding of pink salmon fry on oil-contaminated prey reduced rates of feeding, assimilation of food, and growth, leading to the conclusion that the consumption of oiled prey would increase mortality as a result of decreased growth rates. Carls et al. (1996) also examined the feeding and growth of pink salmon fry in relation to oil-contaminated food: whereas feeding was not suppressed in low-oil treatments, growth was significantly reduced. They concluded that this reduced growth was associated with an increased metabolic demand and reduced assimilation efficiency. Carls et al. (1996) also suggested that pink salmon in Prince William Sound became contaminated primarily through the ingestion of oiled prey after the *Exxon Valdez* spill. These views, however, have been criticized by Brannon and Maki (1996) because the levels of PAHs determined in the waters of Prince William Sound more than 1 week after the oil spill were below those that they considered to be directly harmful to pink salmon and the hydrocarbon content of the experimental diet was unrealistically high. These criticisms are valid in the short term, i.e., after the initial spill event when the more acutely toxic monoaromatic hydrocarbon fractions would have been in their highest concentrations, but do not address the duration of exposure to the oiled environment or feeding on oiled prey and the abundant, yet primarily inferential, field data recording adverse effects (refer to Rice et al. 1996).

Short-term sublethal responses and subsequent survival

In our experiments, the pink salmon adults from the different experimental treatment groups returned in equivalent numbers despite the identification of sublethal responses and the presence of histological effects during exposure to the crude oil WSF (Brand et al. 2000). Sublethal exposure to the WSF of North Slope crude oil resulted in significant stress responses manifested in the liver, kidney, and gill tissues. Although the expression of responses varied between years and treatment groups, most were limited to fry exposed to either the low or high WSF dose. In the absence of any adult return data, it may have been reasonable to propose that the sublethal effects observed in hydrocarbon-exposed fry (i.e., gill, liver, and kidney damage and potential olfactory impediment) would signify a probable loss of health and longevity of exposed individuals. It is therefore conceivable that those fry that exhibited sublethal histological responses to the exposure conditions were the weaker, or more sensitive, individuals within the population and would have succumbed to discriminate and indiscriminate selection pressures in the wild before the more robust individuals. Thus, the oil exposure could have produced higher initial mortality within that fraction of the test populations predisposed to early death.

Pink salmon metabolize hydrocarbons (Rice et al. 1977), and it is possible that fry exposed in our investigation may have rapidly recovered from the 10-day sublethal exposure. Following the 96-h acute lethal bioassay in 1991, four fry surviving dissolved hydrocarbon exposure of 3.1 $mg \cdot L^{-1}$ and all of the 10 fry surviving 1.5 $mg \cdot L^{-1}$ resumed feeding activity after the oil exposure (fry were not fed during the bioassay) and subsequently survived an additional 6 days, at which time the experiment was terminated (I. Birtwell, Department of Fisheries and Oceans, West Vancouver Laboratory, West Vancouver, B.C., unpublished data). Although this result revealed little about the overall health and performance of the fish, it strongly infers a potential for the rapid recovery of some survival behaviors following near-fatal hydrocarbon exposure.

Pink salmon exposed to the WSF of Cook Inlet crude oil (Rice et al. 1977) incurred a rapid increase in tissue hydrocarbons followed by a return to baseline levels despite continued oil exposure. Rapid excretion of tissue hydrocarbons upon release of the fry in our experiment, coupled with metabolism of residual hydrocarbons, could have afforded oil-exposed fish protection from long-term consequences following the sublethal hydrocarbon exposure. Rice and Thomas (1989) found that pre-exposure of pink salmon fry to naphthalene

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(and, to a lesser extent, toluene) activated a mixed-function oxidase system that conveyed an enhanced survival benefit during subsequent acute hydrocarbon exposure, but they suggested that the higher energy utilization necessary for hydrocarbon metabolism could affect other physiological and behavioral functions, including swimming performance, plasma cortisol, and, ultimately, growth. This opinion was also expressed by Carls et al. (1996) after studies that assessed the effects of oil-contaminated food on the growth of pink salmon and by Willette (1996), who assessed the effects of oil spillage from the *Exxon Valdez* on pink salmon in Prince William Sound. Furthermore, Mortenson et al. (1991) deduced that the growth of juvenile pink salmon was significantly related to the survival to adulthood and suggested that predator abundance may have caused annual variations in this relationship. We did not, however, determine a consistent significant dose-dependent effect of crude oil WSF on the growth of pink salmon fry before their release.

Concern over the potential for exposure to benzene (a primary constituent of the WSF of North Slope crude oil present in our investigation) to result in decreased chemosensory reception, with possible consequences to homing, feeding behavior, and avoidance of predators, was expressed by Babcock (1985). However, in our experiment, there was no implication of any long-term effects on homing ability, albeit 17–18 months after exposure to the crude oil WSF. There was also no evidence that the homing of sockeye salmon (*Oncorhynchus nerka*) smolts and adults, which had to swim through *Exxon Valdez* "oil-tainted" areas, was directly affected (Spies et al. 1996). It is possible that adult sockeye salmon swimming through Prince William Sound avoided exposure to spilled oil because of their responses to environmental migration cues that promoted the occupancy of deeper waters (Birtwell et al. 1994). It is unlikely, however, that some sockeye salmon smolts would have escaped exposure to the spilled oil because of their tendency to occupy surface waters, at least during the early part of their estuarine and marine life (Beak Consultants Ltd. 1981).

Exxon Valdez oil spill

The difficulty in determining the extent and significance of the effects of spilled oil on salmonids in the natural environment is exemplified by the studies conducted after the spillage of oil from the *Exxon Valdez* (Wells et al. 1995; Rice et al. 1996). The results of our study, which attempted to assess the effects of the WSF of crude oil on populations of pink salmon (under much less trying circumstances), also serve to illustrate the difficulty of such assessments. In addition, the results provide a basis for comparison of the effects of a whole oil spillage event, and persistent PAHs, on pink salmon versus a short-term exposure that used a highly volatile primarily monoaromatic hydrocarbon fraction of crude oil.

Attempts to determine the environmental effects of crude oil spilled from the *Exxon Valdez* were hindered by a lack of prespill data and precise information on the exposure of individual organisms to oil. Reliance was placed on the nature and inferences of sublethal effects at the time of the spill and afterwards and assessments of population-level effects. In addition, adult pink salmon returns to hatcheries that had released pink salmon into oiled and unoiled regions of Prince William Sound provided comparative statistics on the negative effects of oil spillage on survival to adult (Willette 1996). Geiger et al. (1996) estimated that about 1.9×10^6 adult pink salmon failed to return to Prince William Sound as a consequence of oil spillage from the *Exxon Valdez* in 1989. This represented 28% of the potential wild stock production from the southwest portion of the sound in 1990; however, overall losses in the sound were estimated to be less than 2%. Pink salmon returns to two hatcheries in Prince William Sound were variable and ranged from 6.8 to 8.8% in 1987 but were reduced to 4.5 and 4.99% and to 2.08% and 0.94% in 1991 and 1992, respectively, following the oil spill (Geiger et al. 1996). Rice et al. (1996) present compelling evidence and inferences to conclude that the spillage of oil into Prince William Sound had adverse effects on the survival of salmon that were exposed during the early phases of their marine life. These deductions are not surprising, for even though pink salmon have the ability to detect and avoid concentrations of the WSF of crude oil (Rice 1973; I. Birtwell, Department of Fisheries and Oceans, West Vancouver Laboratory, West Vancouver, B.C., unpublished data), it has been shown that juvenile salmon do not always avoid suboptimal or lethal aquatic conditions in surface waters (Birtwell and Kruzynski 1989; Birtwell et al. 1994). At the time of the *Exxon Valdez* oil spill, juvenile pink salmon were feeding in, and "leaping" through, surface sheens in oiled areas of Prince William Sound (Willette 1996). Futhermore, oil globules or sheen was observed in the stomachs of pink and chum salmon (*Oncorhynchus keta*) from oiled sites (Sturdevant et al. 1996), implying no avoidance of the hydrocarbons in surface waters and no decrease in feeding (as revealed in our 10-day exposure to the WSF of crude oil). Thus, the potential for the spilled oil to debilitate individual fish was increased by their behavior to utilize oiled surface water habitats. The consequence of such sublethal exposures is difficult to determine, but it has been demonstrated that even brief sublethal exposures to stressors may inrease the susceptibility to predation (e.g., Kruzynski and Birtwell 1994; Mesa and Warren 1997). Thus, in the wild, and notwithstanding the potential effects of oil on predators, the presence of pink salmon in the vicinity of an oil spill could result in increased mortality of "affected" individuals: the discriminate predation event being associated with the stress-inducing indiscriminate effects of exposure to a pollutant (Brett 1958). Pink salmon in Prince William Sound had an apparent growth reduction in 1989 after the *Exxon Valdez* oil spill, and it was suggested that this may have predisposed them to higher predation (Willette 1996), with the most potential damage occurring in the "critical" nearshore environment (Geiger et al. 1996).

Despite these opinions, observations, deductions, and the postspill reduction in adult returns to hatcheries in Prince William Sound (Willette 1996), Brannon and Maki (1996) "take exception to the conclusion that adult returns to PWS were reduced from oil spill effects, and conclude to the contrary that evidence has demonstrated no measurable impact of oil on pink salmon survival." These diverging opinions illustrate the difficulty in assessing impacts of a stressor in the wild on anadromous fish that are subject to a wide range of selection pressures, and hence, among years, they often return to their natal streams in variable numbers.

Notwithstanding the problems associated with determin-

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ing population-level effects, it is only logical that sublethal effects that reduce the health and performance of individual aquatic organisms must be avoided if healthy populations are to be sustained.

Summary

Pink salmon fry exposed to the WSF of crude oil and released to the Pacific Ocean matured to adulthood and homed to their natal stream in numbers equivalent to untreated control fry. No significant effect of the exposure to the crude oil WSF was evident on the survival of fry to adulthood in this experiment.

All treatment groups incurred typically high mortality after release, and this may have reduced the resolution of any subtle effects of hydrocarbon exposure on survival to adulthood. Furthermore, it was not possible to determine whether hydrocarbon-exposed fry returning 2 years later as adults were individuals that recovered from apparent tissue damage, individuals that never developed those pathologies, or perhaps individuals with some combination of recovery and tolerance.

It is possible that any selective disadvantage experienced by hydrocarbon-treated versus untreated fry in this experiment was relatively short lived from the time of release. In the protected environment of the holding troughs, natural selection pressures were also minimized, and potential differences in the individual "fitness" of fry from each test group could have been masked. All fry in the hydrocarbon treatment groups were exposed to concentrations of the volatile (primarily monoaromatic) hydrocarbon fractions of crude oil; hence, this exposure represented an indiscriminate stress, as defined by Brett (1958), and as such, potentially affected each member of the exposed populations. The expression of that stress was demonstrated in the idiopathic lesions and stress responses of liver, gill, and kidney tissues in hydrocarbon-exposed fry (Brand et al. 2000). However, many of these cytological effects were limited to a fraction of the individuals examined. This suggests that there was considerable individual variation in hydrocarbon tolerance in the test populations. Furthermore, any consequence of those effects and of exposure to other stressors to the survival and maturation of the experimental pink salmon populations was not detectable in this experiment.

The results of our study reinforce the difficulty encountered when attempting to determine the effects of pollutants on the survival of populations of salmonids under field conditions. While the successful completion of their life cycle, as judged by the number of returning adult fish to their natal stream, may be one measure by which we may assess their response to contaminants, the rigors of selection in the wild detract from its use to detect subtle changes because of the variable and low overall survival. However, the same may not be so for populations that are not subjected to intensive nonselective fishing pressures. The survival of some fraction of a population of pink salmon bears testimony to the robustness and fortune of some of these fish, but it is a coarse indicator of environmental health. It remains an imperative to protect the health and fitness of individuals, for they comprise the populations whose numerical status remains a focus of social and economic attention.

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Temperature- and size-dependent growth of larval and early juvenile Atlantic cod (*Gadus morhua*): a comparative study of Norwegian coastal cod and northeast Arctic cod

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Abstract: Norwegian coastal (NC) and northeast Arctic (NA) Atlantic cod (*Gadus morhua*) larvae were reared on live zooplankton to investigate temperature- and size-specific growth. Larval and juvenile growth was temperature and size dependent. Growth in length and weight increased with increasing temperature from 4 to 14°C, with a corresponding reduced larval stage duration. Maximum growth rate occurred at a larval size of 0.1–1.0 mg dry weight, followed by a declining trend during the juvenile stage. The temperature optimum of larval cod fed in excess is estimated to be between 14 and 16°C, with a maximum weight-specific growth potential exceeding 25%·day^{-1}. Temperature- and stock-specific growth curves of dry weight at age are well described by a generalized Gompertz model. A stock-specific difference in mean weight at age was observed, with NC growing better than NA. Neither countergradient latitudinal variation in growth capacity of the two larval cod stocks nor temperature adaptation across latitudes was indicated. A stock-specific difference in weight at length was observed in early juveniles, with NC being heavier than NA. Overall, a positive correlation between temperature and condition level was found. No distinct temperature- or stock-specific differences in survival were observed.

Résumé : Des larves de morue (*Gadus morhua*) provenant des côtes de Norvège (CN) et du nord-est de l'Arctique (NA) ont été nourries de zooplancton vivant dans le but d'étudier leur croissance en fonction de la température et de la taille. La croissance des larves et des juvéniles était fonction de la température et de la taille. La croissance en longueur et en poids augmentait en fonction de la température dans la gamme de 4 à 14°C et il en résultait un raccourcissement correspondant de l'étape larvaire. Le taux de croissance maximum a été noté au poids larvaire de 0,1–1,0 mg (poids sec), qui était suivi d'une tendance à la baisse pendant l'étape juvénile. La température optimale où les larves se nourrissaient en excès a été estimée comme entre 14 et 16°C, le potentiel de croissance pondéral maximum spécifique excédant 25%·jour^{-1}. Les courbes de croissance spécifiques à la température et au stock par poids sec et âge sont bien décrites par un modèle généralisé de Gompertz. Un écart spécifique au stock du poids moyen selon l'âge a été observé, la croissance des larves CN étant supérieure à celle des NA. Il n'a pas été noté de variations contre-gradient en latitude de la capacité de croissance des larves des deux stocks ni d'adaptation à la température en fonction de la latitude. Un écart du poids selon la longueur spécifique au stock a été noté au début du stade de juvénile, les CN étant plus lourds que les NA. De façon générale, il existait une corrélation positive entre la température et la condition. Aucun écart distinct spécifique à la température ou au stock n'a été observé pour la survie.

[Traduit par la Rédaction]

Introduction

Recruitment in most marine fishes is characterized by high fecundity (Mertz and Myers 1996) and subsequent high mortality during early life stages (Bailey and Houde 1989; Houde and Zastrow 1993). Variability in growth and mortality is known to cause fluctuations in recruitment (Houde 1989, 1997*a*, 1997*b*; Pepin and Myers 1991). The growth and mortality of marine fish larvae depend on temperature, body size, and food availability (Houde and Zastrow 1993). From an evolutionary point of view, it is widely argued that growth should be maximized during the early life of fishes; the so-called "growth–mortality" hypothesis (Hare and Cowen 1997). This hypothesis postulates that larger (e.g., Bailey and Houde 1989), faster growing, and more rapidly developing individuals have a higher probability of survival (Hare and Cowen 1997). Thus, rapid growth may reduce the risk of mortality in larval stages (e.g., Miller et al. 1988) by reaching a larger size at any given age.

Temperature influences metabolic processes and, besides prey availability, is the single most important factor that determines growth rates in fish (Brett 1979). Although the influence of temperature on the growth of Atlantic cod (*Gadus morhua*) has been studied for many years (Jobling 1988), knowledge of the temperature- and size-dependent growth of

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larval and early juvenile stages is limited (e.g., Leising and Franks 1999). Laurence (1978) found growth of larval cod to be positively correlated with temperature in the range 4–10°C. Upper lethal temperatures through the yolk-sac stage ranged from 15.5 to 18°C for larvae acclimated to 7–9°C (Yin and Blaxter 1987). A few temperature-dependent models have been developed, simulating the larval and early juvenile growth for cod on Georges Bank (Campana and Hurley 1989; Leising and Franks 1999). However, there are few laboratory studies on the temperature-dependent growth of larval cod (exception Laurence 1978), and the estimation of the temperature effect is thus uncertain (Leising and Franks 1999). Recent mesocosm studies of larval cod (e.g., Blom et al. 1991, 1994*a*; Folkvord et al. 1994) were all influenced by covarying temperature and food conditions.

Few metabolic relationships are independent of size (Brett 1979). Growth is no exception and it decreases with increasing body size (Jobling 1988; Brander 1995). Although considerable variation in weight-specific growth rate (G) has been reported, with G ranging from <1 to 79%·day^{-1} (Houde and Zastrow 1993), fish larvae are characterized by high potential G relative to larger fish. Growth rates exceeding 20%·day^{-1} have previously been reported for larval cod (e.g., van der Meeren et al. 1994). Temperature optima (T_{opt}) for maximum growth (G_{max}) of fish may show an ontogenetic shift, with larvae and juveniles often having a higher T_{opt} for growth than larger conspecifics (McCauley and Huggins 1979; Jobling 1994). However, it has been suggested that yolk-sac larvae of cod have lower T_{opt} than juveniles and adults (Jobling 1988).

Throughout the area of distribution in the North Atlantic, the numerous cod stocks inhabit regions subject to a wide range of environmental conditions (Brander 1995). Differences among Norwegian coastal (NC) and northeast Arctic (NA) Atlantic cod in growth performance (van der Meeren et al. 1994; Svåsand et al. 1996), genetic (e.g., Møller 1968), meristic, and otolith characteristics (e.g., Rollefsen 1934) have been demonstrated. However, considering the economic and ecological importance of cod, links between genetic characteristics of a specific cod population and growth performance under varying environmental conditions are generally unknown (Svåsand et al. 1996).

The purpose of this study was to characterize temperature- and size-dependent growth of cod larvae and juveniles. An age- and temperature-mediated growth model has been developed, based on the Gompertz equation (Zweifel and Lasker 1976). NC and NA were reared together under identical conditions at temperatures from 4 to 14°C, and the growth and survival patterns of the two stocks were compared. The study was motivated by the hypothesis that the growth patterns of the stocks reflect temperature adaptation across latitudes and that a countergradient latitudinal variation in the growth capacity of the larval cod stocks (i.e., that the genetic capacity for growth varies inversely with the length of the growing season across a latitudinal gradient; Conover et al. 1997) is expected.

Materials and methods

Biological material

NC and NA eggs were naturally spawned in pens (120 m^3) and tanks (5 m^3) in early spring during two seasons (5 April 1995 and 14 March 1996) at the Parisvatnet, Øygarden, and Austevoll aquaculture research stations in western Norway. The broodstocks, 40–100 fish in each population, had been kept in captivity for about 3 years, with the exception of the wild-caught NC spawners utilized in 1996. The NA broodstock originated from wild fish captured in the Lofoten area in northern Norway (68°N) during the 1992 spawning season (Svåsand et al. 1996), and the NC stock originated from western Norway (60°N). The fertilized eggs, a sample of 0.3 L of each stock, were incubated separately in 70-L aerated black conical tanks at 7.3–7.9°C and 32.9–33.8‰ salinity at the Bergen High Technology Center. In both seasons, 50% hatching occurred 12 days after fertilization, referred to as day 0 of larval age.

Experimental design

Two experiments with similar design were undertaken in 1995 and 1996. Initial stocking densities were 1400 larvae, 700 NC and 700 NA, per tank. The 2-day-old yolk-sac larvae were individually counted and randomly distributed into replicate green square fiberglass tanks of 500 L. In 1995, the fish were reared for 8 weeks at three different temperatures (±SD), 4.1 ± 0.2, 8.0 ± 0.1, and 12.0 ± 0.2°C, and in 1996 at 6.1 ± 0.1, 10.0 ± 0.2, 12.0 ± 0.3, and 14.1 ± 0.2°C. Another 100 larvae of each stock were kept in separate 5-L pails as viability groups at the respective temperatures. All larvae were acclimated to the rearing temperatures for 1 day, except for the fish at 12 and 14°C in the 1996 experiment, where the temperature was gradually increased over a period of 5 days. In order to distinguish NC from NA in 1995, we marked the otoliths of the latter stock with alizarin complexone (100 mg·L^{-1} for 24 h) 2 days before hatching, whereas in 1996 the NC larvae were marked (Blom et al. 1994*b*).

Feeding conditions

Larvae and juveniles were fed live natural zooplankton one to five times a day. Larvae <12 mm standard length (SL) were offered a fraction of zooplankton ranging from 80 to 250 µm, juveniles in the range of 12–15 mm were given plankton 80–350 µm, and fish >15 mm were fed zooplankton varying in size from 80 to 2000 µm (mainly copepods). Zooplankton density was sampled daily in each tank (before feeding), and the plankton were counted and categorized into six main groups: small tintinnids, rotifers, and nauplii and the larger calanoid, cyclopoid, and harpacticoid copepodites and copepods. Beginning on day 3, all tanks received an equal amount of zooplankton, more of which was later added to maintain a similar prey density above 1000 individuals·L^{-1} in each rearing unit. Cultivated algae (2–4 L of each species), *Isochrysis galbana* and *Rhodomonas baltica*, were added to each tank three to seven times a week. Tintinnids and rotifers dominated the supplied plankton early in the 1995 experiment, whereas copepod nauplii later became most abundant. The zooplankton diversity in 1996 was initially dominated by copepod nauplii and thereafter by copepodites as fish larvae started to metamorphose. The prey density in tanks decreased as the juveniles grew beyond 15 mm and preyed mainly upon larger copepods.

Rearing conditions

A simulated natural light regime for the latitude of Bergen (60°25′N) was utilized. Maximum photoirradiance in the surface of the water was 1.8–3.5 µE·m^{-2}·s^{-1}. Temperature was measured twice a day throughout the experiments. The water was gently aerated to reduce the patchiness of the prey and larvae. Oxygen concentration (percentage and milligrams per litre) was recorded daily and remained above 70% saturation, while salinity ranged from 30.9 to 33.5‰. Gas saturation, monitored on day 15 in 1995, indicated nitrogen supersaturation (105–107%) in the rearing tanks at

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Table 1. Mean egg and larval sizes of NC and NA in 1995 and 1996.

	Stock			
Year	NC	NA	*n*	*p*
1995				
ED (mm)	1.27 (0.06)	1.31 (0.06)	100	<0.001
SL (mm)	4.23 (0.17)	4.24 (0.15)	70	ns
MH (mm)	0.24 (0.01)	0.25 (0.01)	70	<0.001
DW (mg)	0.027 (0.005)	0.029 (0.008)	20	ns
1996				
ED (mm)	1.40 (0.07)	1.37 (0.06)	100	<0.001
SL (mm)	4.68 (0.16)	4.82 (0.28)	70	<0.001
MH (mm)	0.28 (0.01)	0.27 (0.02)	70	ns
DW (mg)	0.034 (0.006)	0.033 (0.005)	20	ns

Note: *p* values refer to *t* tests comparing stocks. Egg diameter (ED) was measured on day 1 postfertilization. Standard length (SL), myotome height (MH), and dry weight (DW) of yolk-sac larvae were measured on day 2 posthatch. Values in parentheses represent the SD of the estimated parameters, and ns indicates $p > 0.05$.

12°C. The bottom of each tank was siphoned daily from day 14, and dead larvae and juveniles were removed and counted. Water exchange was about 2%·day^{-1} during the first 14 days after larval stocking and was increased to 5–30%·day^{-1} (depending on the biomass) throughout the experiments.

Sampling procedure

Initial samples of 70 yolk-sac larvae were taken on day 2 from each stock tank for estimation of live SL and myotome height (MH) under a dissecting microscope (Wild Heerbrug). Dry weight (DW) was recorded for 20 of these larvae. Another 30 larvae were routinely sampled in each tank once a week at daylight for similar measurements. The fish were transferred individually into marked vials, killed in liquid nitrogen, and stored at –80°C for subsequent otolith and DW analysis. The lapilli were removed and mounted on glass slides using clear varnish. Both lapilli were checked for alizarin marks and classified as either NC or NA using a fluorescence microscope (Zeiss Axioscope) at 200× magnification. The larvae or juveniles ($n = 2172$) were subsequently dried (60°C for 24 h or until stable) and weighed on a Sartorius microbalance (±1 µg). Lapilli were selected for a number of reasons (Meekan and Fortier 1996), and it was possible to detect the alizarin mark without further polishing.

With a few exceptions the larvae and juveniles sampled were analyzed for otoliths and their DW recorded. At 6, 10, and 14°C, only 20 of the 30 sampled larvae and juveniles from each replicate were analyzed and the remaining larvae were retained for other purposes. Another 3.8% ($n = 95$) of the sampled larvae and juveniles were excluded due to missing data on either otoliths or DW. Three tanks were terminated before the 1995 experiment ended due to high mortality, probably caused by nitrogen supersaturation (both replicates at 12°C on day 21 and one replicate at 8°C on day 28). Data from the 12°C group for that year were excluded from further analysis, since data from this temperature regime were available from the 1996 experiment. In 1996, one tank at 14°C was terminated on day 35.

Data analysis

The relationship between larval stage duration (D, days) and temperature (T, degrees Celsius) was described by a power function as

$$(1) \qquad D = aT^{-b}$$

where a and b are estimated constants (e.g., Houde and Zastrow 1993). Larval stage specific survival was estimated according to Beyer (1989) and Houde (1997*a*):

$$(2) \qquad N_1 = N_0\left(\frac{DW_1}{DW_0}\right)^{-\frac{M}{G}}$$

where N_1 and N_0 are the numbers of larvae alive at the end and beginning of the larval stage, respectively, bounded by dry weights $DW_1 = 1.3$ mg, mean DW at onset of metamorphosis, and DW_0, the initial larval weight (day 2). Parameter M is the daily instantaneous mortality rate and G is the daily weight-specific growth rate. The fish alive at the termination of the experiments were counted and sorted with respect to stock. Estimated total survival for the respective treatments was corrected for sampling mortality (i.e., estimated as the mean of the number of fish alive at termination of the experiment and the number of individuals alive at termination plus the number of larvae and juveniles sampled). Survival during the larval stage was estimated similarly, corrected for natural mortality recorded during the juvenile stage. Daily weight-specific growth rate G was calculated according to Houde and Schekter (1981):

$$(3) \qquad G = 100(e^g - 1)$$

where the instantaneous growth coefficient g is

$$(4) \qquad g = \frac{(\ln DW_2 - \ln DW_1)}{(t_2 - t_1)}$$

and DW is average dry weight (milligrams) on days t_1 and t_2, respectively. The shift in mean specific growth rate G with temperature T is described by the following regression:

$$(5) \qquad G = a + bT + cT^3$$

where a, b, and c are estimated constants (Jobling 1988). The length–weight relationships of ln-transformed data were described by the general allometric function

$$(6) \qquad \ln DW = \ln a + b\ln SL$$

where a and b are constants (e.g., Pepin 1995). A second-order polynomial was fit to the residuals to test for departure from log-linearity (Pepin 1995). Only one third of the length–weight relationships analyzed were slightly nonlinear. Temperature- and stock-specific growth curves of DW with age were described by the Gompertz equation:

$$(7) \qquad \ln DW(t) = C + Ae^{K(1-e^{-\alpha t})}$$

with DW (milligrams) at age t (days) and where C and A are constants, K is an expression of growth rate, and α is the rate of exponential decay (Zweifel and Lasker 1976). Parameters of the equation were estimated by nonlinear estimation. A generalized temperature-dependent growth model was created by substituting α with a linear temperature function and pooling data from the six different temperature regimes:

$$(8) \qquad \alpha = a + bT$$

where a and b are constants and T is temperature. The procedure for further modifications (initiation, substitution, and reparameterization) of the Gompertz growth model is outlined in the Appendix. Finally, the current model was tested with independent growth data of larval cod from two mesocosm studies (Folkvord et al. 1994; Blom 1995).

Initial stock differences in egg diameter (ED) and larval DW, SL, and MH within a season were tested using Student's t test (Zar 1984), whereas a two-way ANOVA was employed for a similar ini-

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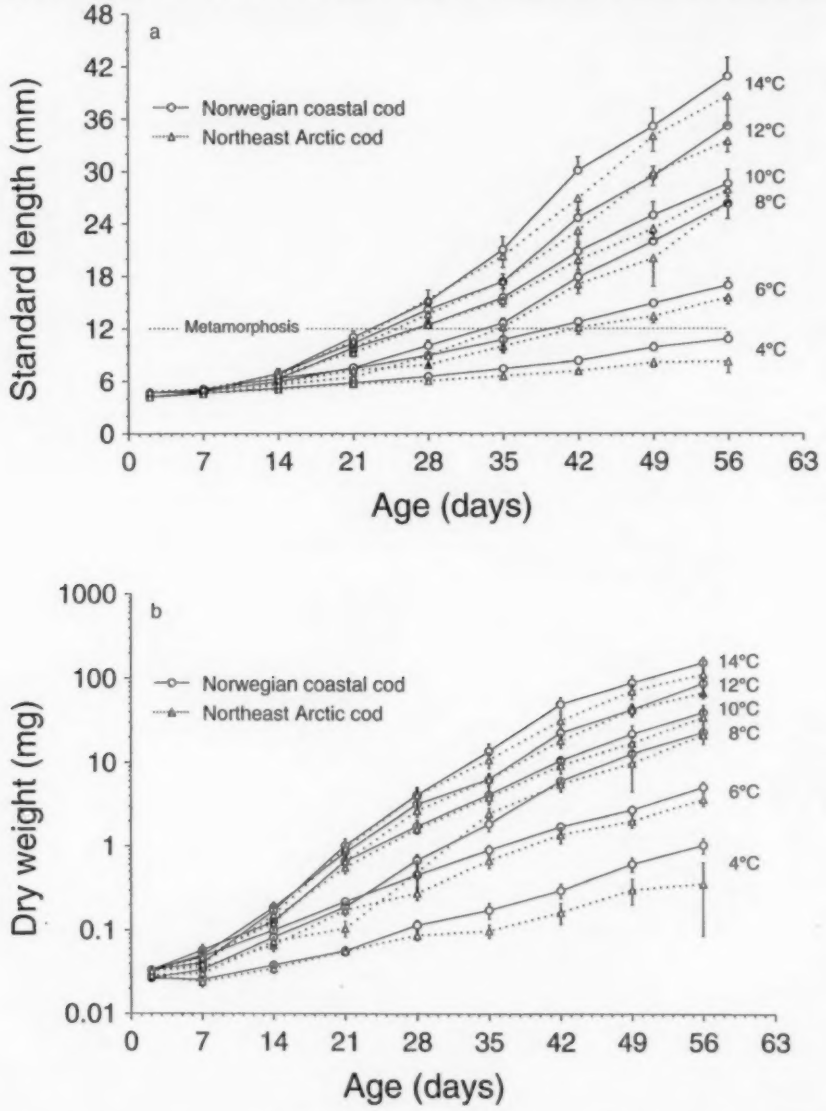
Fig. 1. Mean size at age, (*a*) SL and (*b*) DW, of NC and NA reared at different temperatures. Vertical lines indicate 2 SE of the mean.



tial comparison between stock and seasons. For further growth comparison, data of replicates and seasons were combined (DW differed in 11% of the replicate *t* tests carried out). A two-way ANOVA was employed to estimate the effects of temperature and stock on mean DW and SL during the experimental periods. The data were checked for normality and the variance tested for heterogeneity using Hartley's F_{max} test. Data on DW and SL were ln transformed (Zar 1984). Significant ANOVAs were followed by a Tukey's (HSD) multiple comparison test to determine differences among treatments. In cases of significant interactions between factors, the treatments were split up and one-way ANOVAs were employed.

The effects of temperature on mean stock-specific growth rates were described by multiple regressions. Multiple comparisons of the length–weight regressions between treatments were tested with ANCOVA (Zar 1984). The ANCOVA was employed for overlapping size ranges: larvae (SL ≤ 10 mm, temperatures 4, 6, 10, 12, and 14°C) and juveniles (SL ≥ 14 mm, temperatures 10, 12, and 14°C). Larvae reared at 8°C differed significantly in slope from the other groups and separate stock-specific regressions were computed for this treatment. Effects were considered significant at a probability level of $\alpha < 0.05$.

Results

Growth

Effects of temperature, stock, and fish size

Initial larval DW (day 2) of the two stocks was not significantly different (two-way ANOVA, $p = 0.76$). However, ED of both stocks was significantly smaller (one-way ANOVA, $p < 0.001$) in the late-spawned batches in 1995 compared with the earlier spawned egg batches used in 1996. Conse-

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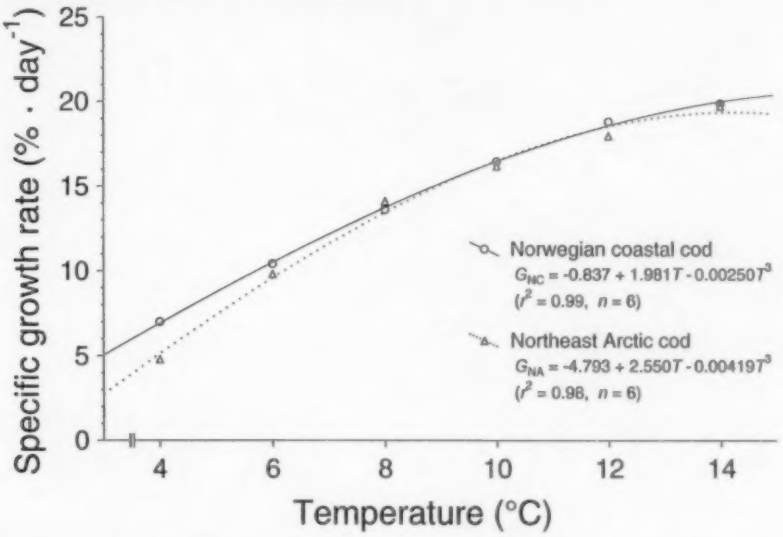
Fig. 2. Effects of temperature on mean weight-specific growth rates of NC and NA during the larval stage (SL ≤ 12 mm). Each point represents the mean growth rate of fish at the respective temperature.



quently, the initial larval DW was higher in 1996 (two-way ANOVA, $p < 0.001$) (Table 1).

Somatic growth in length and weight increased successively with increasing temperature from 4 to 14°C (Fig. 1). The final mean DW of all groups differed significantly (two-way ANOVA, $p < 0.001$) with the exception of the juveniles reared at 12 and 14°C ($p = 0.06$). Larval stage duration declined rapidly as temperature increased. Larvae reared at 14°C reached the onset of metamorphosis (12 mm SL) after 23 days compared with more than 56 days for fish at 4°C. The relationship between larval stage duration D and temperature was described by the following function: $D = 132.0T^{-0.685}$ ($r^2 = 0.97$, $n = 9$) for NC and $D = 152.9T^{-0.741}$ ($r^2 = 0.98$, $n = 9$) for NA. An increase in temperature from 6 to 12°C generated a 17-fold higher DW after 8 weeks. A stock-specific difference in mean DW at age was observed, with NC generally growing significantly faster than coreared NA from day 7 onwards (two-way ANOVA, $p < 0.001$). Mean DW and SL of NA were at most 27.0 and 7.4% lower, respectively, than those of NC at the end of the experiments at any given temperature.

Larval and juvenile weight-specific growth rates G were temperature and size dependent. Mean G increased with increasing temperature from 4 to 14°C. The T_{opt} for G_{max} of larval cod fed in excess was estimated at 16.2 and 14.2°C for NC and NA, respectively (Fig. 2). The G also increased transiently with increasing larval weight at all temperatures (Fig. 3). The G reached a peak at a larval size of 0.1–1.0 mg DW and then declined during the juvenile stage. Estimated G_{max} in both NC and NA larvae exceeded 25%·day^{-1}. Temperature had relatively little effect on larval mean G between 10 and 14°C ($Q_{10} = 1.6$, equal for both stocks). The effect of temperature was greater ($Q_{10} = 3.1$ and 3.5 for NC and NA, respectively) at lower temperatures from 6 to 10°C during the larval stage.

Function of age

Larval DW was modeled as a function of age and temperature, expressed as a modification of the Gompertz equation (Appendix, eq. A4):

$$\ln DW(t) = \ln DW_0 - A + A \times \left(\frac{\ln DW_\infty - \ln DW_0 + A}{A} \right)^{(1-e^{-(a+bT)t})}$$

The fit of the data was good for both stocks ($r^2 = 0.95$ and $r^2 = 0.94$ for NC and NA, respectively) (Table 2). Although the initial DW was similar between stocks, predicted DW at age was consistently higher for NC than for NA (Fig. 4). Predicted mean DW of larvae on day 7 ranged from 35 to 65 µg and largely overlapped the range of the observed data, 24–56 µg (Table 3). As the experiments proceeded, the estimated values tended to be higher for the larvae reared at 4°C compared with the observed data, whereas the opposite occurred at 6°C and during the latter part of the experiment at 8°C. An estimated G_{max} of 26%·day^{-1} on days 18–20 for both stocks reared at 14°C agrees with the observed data (Figs. 3 and 5). Furthermore, the predicted peak in G of 8–9%·day^{-1} on days 51–56 for the stocks at 4°C is in accordance with our observations. The current model for the NC stock slightly underestimated the growth for larvae <1.5 mg DW, tested with independent data sets of larval cod from both of the two mesocosm studies (Fig. 6). The difference between the observed and estimated larval weight constituted <1–4 days of growth at the respective temperature.

Length–weight relationships

The length–weight relationships of cod indicate positive allometric growth at both the larval and juvenile stages ($b > 3$). An ontogenetic shift in the length–weight ratio occurred at the onset of metamorphosis (Fig. 7). Even though the early juveniles continued to increase in weight at a greater rate than in length ($b = 3.66$), the relative ratio decreased compared with the larval stage ($b = 4.01$ and $b = 4.14$ for NC and NA, respectively). A stock-specific difference in

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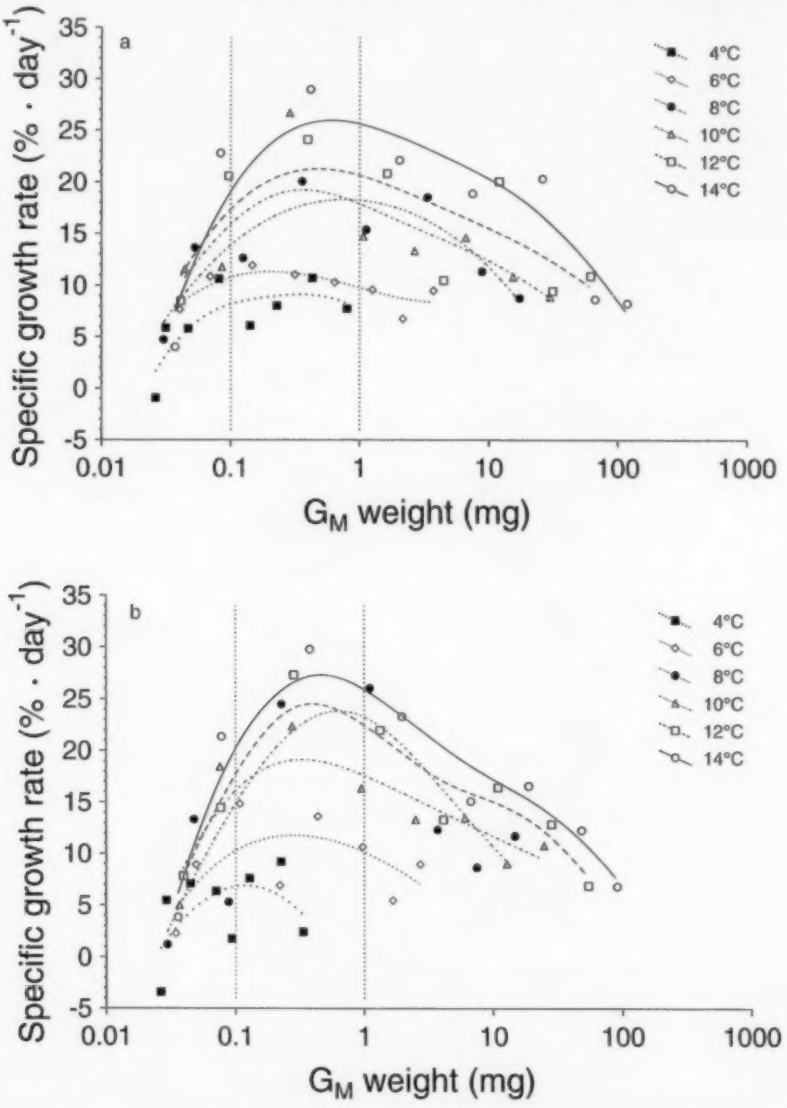
Fig. 3. Weight-specific growth rate versus geometric mean DW of (*a*) NC and (*b*) NA larvae and juveniles reared under different temperature regimes. The fitted lines (least squares) indicate the general trends in growth. Data from replicates are combined.



weight at length was observed in early juveniles, with NC being heavier than the NA stock (ANCOVA, $p < 0.001$). Larval cod significantly differed in stock-specific slopes with regard to weight at length ($p < 0.05$) and were not comparatively tested. However, within both stocks the weight at length of larvae and juveniles significantly increased with temperature (ANCOVA, $p < 0.001$), indicating a positive correlation between temperature and condition level (Table 4). Larvae reared at 12°C were 49% heavier than larvae of similar length at 4°C. Only larvae reared at 14°C differed from this trend, showing an intermediate condition level.

Mortality and the *M*/*G* ratio

Larval stage-specific mortality *M* was high and variable and ranged from 0.035 to 0.109·day^{-1} for NC and from 0.019 to 0.110·day^{-1} for NA (Table 5). High *M* was observed during three critical periods: the first day after the larvae had been transferred to the rearing tanks, at the onset of first-feeding, and during development and filling of the swim bladder at a larval size of 6–10 mm SL. No immediate increase in *M* was recorded in the viability groups, with survival ranging from 80 to 98% (NC) and from 88 to 100% (NA) on day 7. The *M*/*G* ratio during the larval stage ranged from 0.23 to 0.58 for NC and from 0.19 to 1.12 for NA at the different temperatures. The estimated mean *M*/*G* ratio was <1.0 for both stocks, averaging 0.39 and 0.49 for NC and NA, respectively, showing a net biomass gain (Table 5). Subsequent *M* of fish >12 mm mean SL was negligible and comprised <2.6% of the initial number of larvae in the tank.

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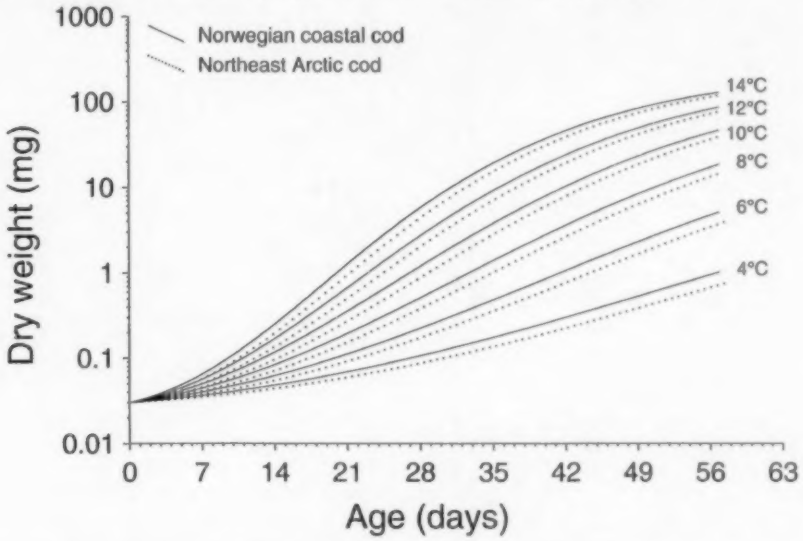
Table 2. Summary of the initial values and growth parameters generated by the Gompertz equation (Appendix, eq. A4).

			Parameter estimation				
Stock	DW_0 (mg)	DW_∞ (mg)	A	a	b	n	r^2
NC	0.030	250	0.271 (0.030)	0.0065 (0.0006)	0.0044 (0.0001)	1072	0.95
NA	0.030	250	0.198 (0.022)	0.0061 (0.0007)	0.0044 (0.0001)	1020	0.94

Note: The parameters are based on DW measurements of NC and NA larvae and juveniles during their first 8 weeks of life, reared at six constant temperatures in the range of 4–14°C. Initial values DW_0 and DW_∞ are fixed according to the range of the data set. Values in parentheses represent the SE of the estimated parameters.

Fig. 4. Modified Gompertz growth curves of ln DW at age for NC and NA larvae and juveniles reared at six constant temperatures from 4 to 14°C. Equation and parameter estimates are given in the text and Table 2, respectively.



Taking both experiments into account, larval stage specific M varied with respect to year, temperature, and stock, without showing any distinctive trends (Table 5).

Discussion

Temperature may be the single most important factor determining the growth rates of early life stages of fish in the field (Houde 1989; Blaxter 1992). The growth rate of cod larvae in this study increased at temperatures from 4 to 14°C (e.g., an increase in temperature from 6 to 12°C generated a 17-fold higher DW after 8 weeks). Successful rearing of cod larvae at 10°C and a positive correlation of G with temperature, in the range of 4–10°C, have previously been reported (Laurence 1978). Estimated values of Q_{10} in the range of 3.1–3.5 for G of larval cod between 6 and 10°C in our experiment are higher, although still comparable with a value of Q_{10} of 2.5 for larval cod between 7 and 10°C in Laurence (1978).

Estimated T_{opt} for G_{max} of larval cod fed in excess is in the range of 14–16°C. The T_{opt} is high and close to the reported upper lethal temperature, ranging from 15.5 to 18°C, of yolk-sac larvae of cod acclimated to 7–9°C (Yin and Blaxter 1987). Furthermore, the estimated T_{opt} is higher than previously suggested for larvae in the field, 5.9°C (Campana and Hurley 1989), but close to what has been reported for juveniles and larger cod within the size range 250–2000 g, 13.5°C (Jobling 1988). Previous efforts to model age- and temperature-dependent growth in field have been influenced by covarying temperature and food conditions. Restricted prey availability can possibly explain the relatively low temperature estimated for G_{max} of cod larvae in the field given by Campana and Hurley (1989). The term "optimum temperature for growth" therefore should only be used where fish are feeding close to maximum ration (R_{max}), since restricted food intake will have a marked influence on G_{scope} at any given temperature (Brett 1979; Jobling 1994), effectively reducing T_{opt} at lower rations (Brett 1979).

Cod larvae and juveniles demonstrated a dome-shaped relationship between weight-specific growth rate and body size. Growth rate initially increased with larval size at all temperatures, peaked at a premetamorphic size of 0.1–1.0 mg DW, and then declined during the juvenile stage. The estimated G_{max} for both NC and NA larvae exceeded 25%·day^{-1}. These observations are in accordance with some earlier mesocosm and enclosure studies on cod (van der Meeren and Næss 1993; van der Meeren et al. 1994). Average values of G in the range of 18.1–22.3%·day^{-1} have been reported for larval cod reared for 46 days in enclosures at sea temperature increasing from 7°C initially to 16°C at termination (van der Meeren et al. 1994). An ontogenetic increase in digestive capacity offset by metabolic cost of growth is further expected to result in a peak or maximum growth potential at a certain body size (van der Meeren

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Table 3. Size-at-age (dry weight, mg) of NC and NA larvae and juveniles reared at different temperatures.

Stock	Age (days)	4°C			6°C			8°C			10°C			12°C			14°C		
		Obs	Est	*n*	Obs	Est	*n*	Obs	Est	*n*	Obs	Est	*n*	Obs	Est	*n*	Obs	Est	*n*
NC	7	0.025	**0.037**	34	0.048	**0.040**	14	0.032	**0.044**	24	0.056	0.050	10	0.048	**0.057**	22	0.037	0.065	4
	14	0.036	**0.048**	32	0.098	**0.062**	13	0.081	0.084	21	0.104	0.117	16	0.168	0.169	28	0.151	**0.251**	13
	21	0.054	**0.069**	31	0.217	**0.112**	11	0.164	0.195	37	0.649	**0.357**	17	0.787	0.671	29	0.935	**1.264**	17
	28	0.109	0.107	28	0.452	**0.225**	11	0.516	0.514	40	1.558	1.195	17	3.088	**2.702**	28	3.805	**5.731**	9
	35	0.155	0.176	21	0.883	**0.486**	14	1.738	1.393	15	3.863	3.779	13	5.808	**9.139**	25	12.199	**19.227**	17
	42	0.250	0.303	40	1.682	**1.069**	23	5.972	**3.593**	21	10.388	10.285	22	21.372	24.075	34	48.508	46.335	8
	49	0.503	0.536	42	2.663	**2.307**	19	12.358	**8.374**	26	19.797	23.285	20	39.687	49.593	37	84.936	84.571	11
	56	0.714	**0.956**	53	4.953	4.729	14	22.798	**17.242**	24	36.942	44.043	18	83.611	83.157	36	148.269	125.714	13
NA	7	0.024	**0.035**	19	0.036	0.038	19	0.030	**0.041**	33	0.041	**0.046**	30	0.045	**0.051**	38	0.038	**0.057**	35
	14	0.034	**0.044**	23	0.060	0.055	27	0.069	0.072	36	0.121	0.097	24	0.105	**0.136**	32	0.130	**0.197**	27
	21	0.055	0.060	24	0.161	**0.092**	29	0.094	**0.154**	23	0.515	**0.274**	22	0.571	0.505	31	0.824	0.946	8
	28	0.084	0.088	20	0.244	**0.175**	29	0.289	0.385	17	1.548	**0.886**	23	2.350	2.017	32	3.870	4.365	16
	35	0.095	**0.138**	13	0.604	**0.361**	26	2.472	**1.022**	3	3.600	**2.815**	27	5.822	**7.035**	35	10.093	**15.406**	14
	42	0.146	**0.228**	20	1.255	**0.777**	17	5.458	**2.649**	7	8.615	7.900	18	16.368	19.445	26	30.322	**39.277**	12
	49	0.244	**0.390**	18	1.865	1.668	21	8.852	6.317	4	15.737	18.647	20	39.685	42.075	23	69.302	75.250	9
	56	0.277	**0.682**	7	3.299	3.454	26	21.280	**13.443**	5	31.808	36.281	22	65.011	73.633	24	103.891	116.077	6

Note: Observed (Obs) values are retransformed data. Estimated (Est) values from the modified Gompertz equation (Appendix, eq. A4) presented in boldface differ from the 95% confidence interval.

1993). These results are of particular interest because it is not the mortality rate alone that determines stage-specific survival but rather the *M/G* ratio. An increase in *G* alone can act to impart major reductions in stage-specific mortality rate, *M/G*, without concomitant reductions in *M* (Houde 1997*b*). However, the potential for *G* to increase during the larval stage has not been documented for gadid larvae in the field (Houde 1997*b*), although it has previously been described for a few other species, e.g., American shad (*Alosa sapidissima*), striped bass (*Morone saxatilis*), and Pacific herring (*Clupea pallasi*) (Houde 1997*b*). The association of increased *G* with increasing size indicates that better foraging ability is associated with advancing development (Pepin 1991).

Stock-specific differences in mean DW at age were observed, with NC generally growing better than the coreared NA from day 7 onward. A higher *G* among NC (14.4–22.3%·day^{-1}) than among NA larvae (13.6–19.7%·day^{-1}) given identical conditions has previously been reported (van der Meeren et al. 1994) and further documented for juveniles and adult cod of the same origin (Svåsand et al. 1996). Faster somatic growth of the southern NC stock, as compared with NA, through the larval and early juvenile periods did not support the hypothesis of countergradient latitudinal variation in the growth of fishes (Conover et al. 1997). Recently, strong evidence has been provided that the capacity for somatic growth in the juvenile stage of some species, e.g., striped bass, varies genetically among native populations, being inversely correlated with the length of the growing season across a latitudinal gradient (Conover et al. 1997). The observed growth performance of the two cod stocks studied, however, may reflect differences in life history strategy. In nature, although growing slower and mature at an advanced age (Godø and Moksness 1987), NA gains a considerable greater maximum size in length and weight than NC. Furthermore, the growth pattern of the two cod stocks did not reflect any temperature adaptation across latitudes, e.g., high-latitude forms display faster growth at low temperatures, while southern forms grow faster at high temperatures (Lonsdale and Levinton 1985). Except for the occurrence of NC larvae growing better than the NA larvae at 4°C, which is difficult to explain with respect to geographical distribution, the differences in *G* and G_{max} between the two stocks are minor relative to the significance of temperature.

Due to the setup of the spawning tanks, i.e., mixed spawning groups, it was not feasible to relate the larvae to individual spawners. Effects of maternal origin cannot be separated from other genetic stock effects on the basis of the present data. Size and condition factor of the females broodfish are known to influence egg and larval size of cod at hatching (e.g., Blom et al. 1994*a*). Nevertheless, the average growth rates during the larval and early juvenile stages did not differ between progeny of large and small females (Blom et al. 1994*a*).

Weight at length for larvae and juveniles of both stocks increased with temperature, indicating a positive correlation between temperature and condition level. Larvae reared at 12°C were 49% heavier than larvae of similar length at 4°C. The maintenance ration R_{maint} increased with temperature (e.g., Brett 1979) and so did *G* within the temperature range 4–14°C in this study. Food was not a limiting factor; conse-

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Fig. 5. Estimated weight-specific growth rate at age for NC and NA larvae and juveniles reared at temperatures from 4 to 14°C. Values for growth rate are estimated from the derivative of the modified Gompertz equation (Appendix, eq. A6).

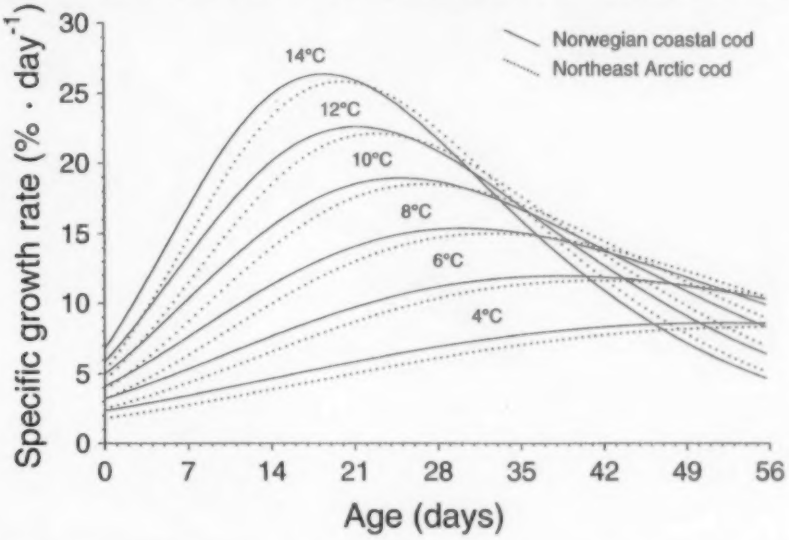
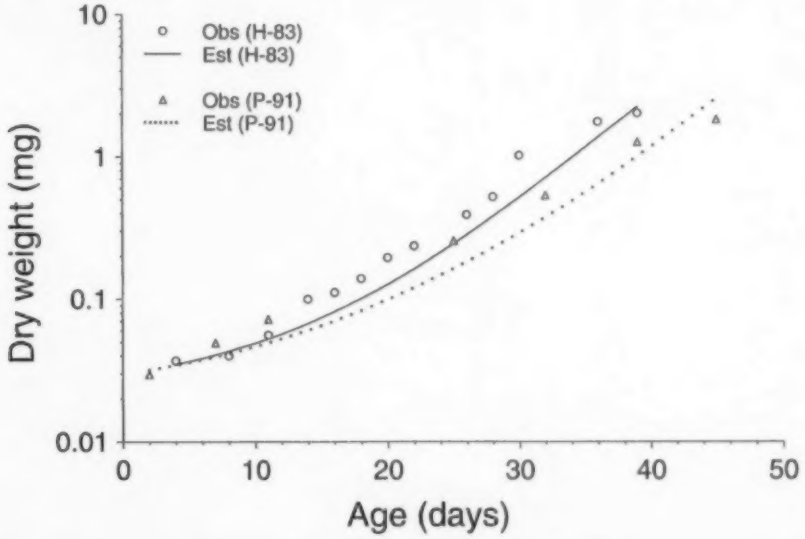


Fig. 6. Current modified Gompertz model for NC, tested with independent growth data of larval cod from two mesocosm studies, Hyltropollen 1983 (H-83) (Folkvord et al. 1994) and Parisvatnet 1991 (P-91) (Blom 1995). Observed (Obs) and estimated (Est) values are presented.



quently, consumption increased with increasing temperature. Larvae and juveniles were not starved or gutted before weighing. Nevertheless, the differences in the condition level of the fish cannot be explained by the difference in weight of the stomach contents alone. Evidence of a stock-specific difference in weight at length was found in early juveniles, with NC being heavier than NA. Differences in body form and energy allocation among juveniles and adults have been documented, with NA having lower condition factor and hepatosomatic indices than NC (Svåsand et al. 1996).

Combining the data across both years, temperature- and stock-dependent growth of larval and early juvenile cod was well described by Gompertz growth curves. The Gompertz model is suitable when an inflection in the growth trajectory is evident (Zweifel and Lasker 1976) and has previously been employed to predict larval growth of fish, e.g., cod (Bolz and Lough 1988). The Gompertz curve is asymmetrical about the inflection point and approaches the asymptote more gradually than the symmetrical logistic curve (Kaufmann 1981) and more resembles the growth pattern of the early life stages of cod. However, to generalize the model, the parameter α had to be substituted with a temperature

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Fig. 7. Length–weight relationships of NC and NA. Generalized common equations for larvae (larv) of overlapping size (SL ≤ 10 mm; temperatures 4, 6, 8, 10, 12, and 14°C) and early juveniles (juv) (SL ≥ 14 mm; temperatures 10, 12, and 14°C) are presented.

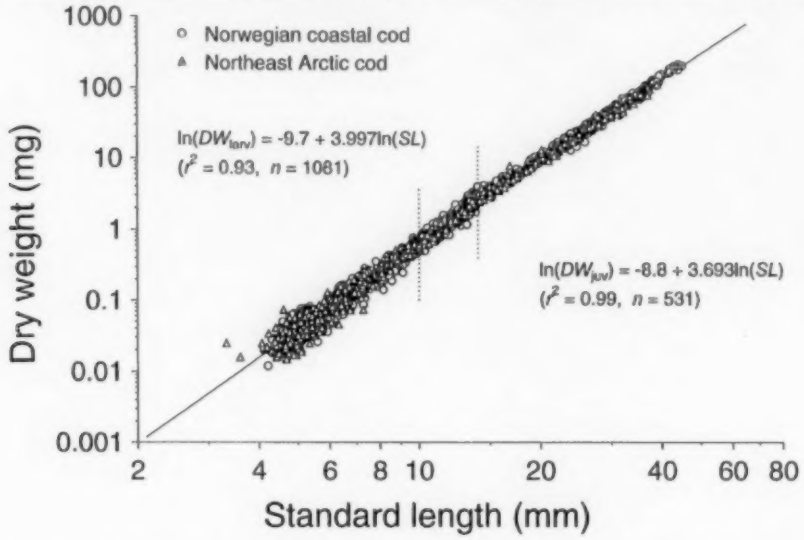

Table 4. Length–weight relationships of NC and NA larvae and juveniles of overlapping size ranges.

Stage	Stock	Temperature (°C)	Range in length (mm)	Median length (mm)	Intercept (*a*)	Slope (*b*)	*n*
Larvae	NC	4	4.1–10.0	6.5	–9.85	4.01	228
	NC	6	4.6–10.0	6.7	–9.60	4.01	53
	NC	8	4.5–9.8	6.3	–9.32	3.76	96
	NC	10	4.6–9.9	6.6	–9.52	4.01	38
	NC	12	4.4–9.9	6.4	–9.45	4.01	60
	NC	14	4.7–9.9	7.0	–9.57	4.01	21
	NA	4	4.4–10.0	5.9	–10.07	4.14	142
	NA	6	4.3–10.0	6.9	–10.00	4.14	121
	NA	8	3.3–9.6	5.4	–8.52	3.33	100
	NA	10	4.5–10.0	6.1	–9.92	4.14	72
	NA	12	4.4–10.0	5.5	–9.83	4.14	87
	NA	14	4.6–8.5	5.3	–9.97	4.14	63
Juveniles	NC	10	14.5–35.0	23.3	–8.71	3.66	71
	NC	12	14.0–40.0	26.5	–8.63	3.66	148
	NC	14	14.3–45.0	29.8	–8.58	3.66	56
	NA	10	14.0–34.5	21.4	–8.71	3.66	82
	NA	12	14.2–39.0	22.8	–8.68	3.66	120
	NA	14	14.1–44.5	23.8	–8.66	3.66	54

Note: Parameters of the general allometric model (eq. 6) were estimated and the number of observations (*n*) is provided. Data from replicates are combined. Larvae reared at 8°C significantly differed in slope from the other groups and separate stock specific regressions were computed for this treatment.

function. Furthermore, in order to track growth correctly and avoid serious over- or underestimations, the model was restricted to the range of the data set by defining fixed initial and "infinite" values.

If the temperature increases, the temperature term (eq. 8) will push the Gompertz to its asymptote more quickly, but compared with the model of Campana and Hurley (1989), the expression will not produce a temperature optimum for growth. This is an unrealistic feature, which limits the application of the models to temperatures below the optimum. However, the T_{opt} for G_{max} of both stocks fed in excess was beyond the temperature range studied. Yet, the data basis for a more complex model, including a temperature optimum, does not exist. Further effort should be directed towards construction of growth–ration curves for a broad range of temperatures, simulating restricted food conditions. The previous

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Table 5. Summary of mean daily mortality rates (*M*) and ratios of mortality rates to mean daily specific growth rates (*M/G*) during the larval stage (DW ≈ 1.3 mg), estimated mean final survival (*S*, %) until age 56 days, and number of days to complete larval mortality (CM) in the respective viability groups.

Stock	Temperature (°C)	*M*	*M/G*	*S*	CM
NC	4[a]	0.037	0.53	14.5	19
NC	6	0.035	0.34	25.3	22
NC	8[b]	0.058	0.41	8.7	16
NC	10	0.043	0.26	31.8	16
NC	12	0.043	0.23	35.5	13
NC	14[b]	0.109	0.58	13.6	12
NA	4[a]	0.053	1.12	6.1	29
NA	6	0.019	0.19	45.0	24
NA	8[b]	0.077	0.58	4.9	17
NA	10	0.033	0.21	39.1	16
NA	12	0.052	0.29	26.5	14
NA	14[b]	0.110	0.56	11.0	13

Note: Estimated values of *M* and *M/G* of larvae at 8°C are based on one tank.

[a]Estimated values of larvae age 56 days, DW < 1.3 mg.

[b]Reduced final survival due to one tank being terminated before the experiment ended.

model of Campana and Hurley (1989) was developed for cod and haddock (*Melanogrammus aeglefinus*) grown at varying ambient temperature. Although the present growth models are based on larvae reared under constant temperature conditions, it is possible to integrate growth across a time-varying temperature regime using DW at a given temperature to estimate the corresponding age in days under an alternative temperature regime.

Finally, the predictive capability of the model for the NC stock was assessed through tests with independent growth data of larval cod from two mesocosm studies (Folkvord et al. 1994; Blom 1995). The model slightly underestimated the growth for larvae <1.5 mg DW. Size-dependent mortality or cannibalism occurring in the mesocosms could have resulted in the predicted growth anomalies. Ambient temperature may also differ from the mean daily estimated temperature actually used in the model. The high growth rates obtained in both mesocosms, however, are reflected by the high larval survival (ranging from 25 to 40% to metamorphosis) in these studies (Blom et al. 1994*a*; Folkvord et al. 1994).

From a multispecies perspective, mortality rates and growth rates are highly correlated in the field (Houde 1989; Pepin 1991), with rates often inversely related for single species (e.g., Campana et al. 1989). If mortality rates are specific to particular life periods, then estimating the duration and rates that apply within the period is crucial (Chambers and Leggett 1987). Given that metamorphosis in larval fish tends to be size rather than age related (Chambers and Leggett 1987), the duration of the larval stage or age at metamorphosis declines rapidly as growth rate increases. Larval cod reared at 14°C reached the onset of metamorphosis after 23 days compared with more than 56 days for fish at 4°C. An inverse relationship between stage duration and temperature has been well documented in other species (Laurence 1975). Time to metamorphosis of the winter flounder (*Pleuronectes americanus*, previously *Pseudopleuronectes americanus*) was 49 days at 8°C and 80 days at 5°C (Laurence 1975). According to the "stage duration" mechanism, if juveniles experience a lower mortality rate than larvae, then individuals that develop faster and metamorphose at an earlier age would have a lower probability of mortality compared with individuals that remain longer as larvae (Chambers and Leggett 1987; Hare and Cowen 1997). Recent studies support the hypothesis that fast growth increases the survival of cod during the larval (Meekan and Fortier 1996) and juvenile (Campana 1996) stages.

Mortality rates during the early life stages of fish are generally high (e.g., McGurk 1986). High mortality observed during the first day after the yolk-sac larvae had been transferred to the rearing tanks may partly be explained by stress during handling or as a consequence of variable larval quality or viability. Furthermore, mortality seems to be associated with two more critical periods, larvae that failed to initiate and maintain successful feeding in switching from endogenous to exogenous nutrition and, later, larvae in the size range of 6–10 mm SL that were unable to regulate and fill their swim bladder. This mortality pattern is typical of marine fish larvae and has previously been reported for intensively reared cod larvae (Howell 1984). Cod larvae, in contrast with Atlantic herring (*Clupea harengus*) larvae, appear to be particularly vulnerable to nitrogen supersaturation. Mortality of fish >12 mm mean SL was negligible in the present experiments.

In summary, this study has demonstrated a maximum weight-specific growth potential for larval cod exceeding 25%·day^{-1}. The estimated temperature optima for maximum growth of 14.2 and 16.2°C for larval NA and NC, respectively, fed in excess are higher than previously suggested. Overall, a positive correlation between temperature and condition level was found. The growth rate is markedly size dependent, describing a dome-shaped relationship in both stocks. Growth curves of size at age are well described by a generalized Gompertz model. A stock-specific difference in mean DW at age was observed, with NC growing better than NA. However, neither temperature adaptation across latitudes nor countergradient latitudinal variation in growth capacity of the two larval cod stocks was indicated.

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Appendix

Initiation, reparameterization, and final equations for the modified Gompertz model are as follows. The initial DW_0 and "infinite" DW_∞ values were fixed according to the range of the data set, $DW_0 = 0.030$ mg and $DW_\infty = 250$ mg, respectively:

(A1) $\ln DW_0 = C + A$

and

(A2) $\ln DW_\infty = C + Ae^K$.

Substituting eq. A1 into eq. A2, an equation for parameter K is derived:

(A3) $$K = \ln\left(\frac{\ln DW_\infty - \ln DW_0 + A}{A}\right)$$

and

(A4) $$\ln DW(t) = \ln DW_0 - A + A\left(\frac{\ln DW_\infty - \ln DW_0 + A}{A}\right)^{(1-e^{-(a+bT)t})}.$$

Data were fitted to eq. A4 and the model tracks growth for the first 8 weeks of life. Furthermore, the instantaneous growth coefficient g is expressed as the first derivative of eq. 7:

(A5) $$g = Ae^{K(1-e^{-\alpha t})}\alpha K e^{-\alpha t}$$

or reparameterized with respect to $\ln DW_\infty$ and $\ln DW_0$:

(A6) $$g = \left[A\left(\frac{\ln DW_\infty - \ln DW_0 + A}{A}\right)^{(1-e^{-(a+bT)t})}\right]\left[(a+bT)\ln\left(\frac{\ln DW_\infty - \ln DW_0 + A}{A}\right)e^{-(a+bT)t}\right].$$

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Influence of waterborne cations on zinc uptake and toxicity in rainbow trout, *Oncorhynchus mykiss*

Derek H. Alsop and Chris M. Wood

Abstract: The effects of waterborne cations on ^{65}Zn uptake, Zn toxicity, and relationships with Ca uptake were examined in juvenile rainbow trout, *Oncorhynchus mykiss*, in soft water. Whole-body Zn uptake (waterborne [Zn] = 100 $\mu g \cdot L^{-1}$ = 1.5 μM) was greatly reduced by a variety of cations. This reduction was directly related to the concentration of positive charges, regardless of which ion carried that charge. Thus, 1.0 mM Na^+, K^+, NH_4^+, and *N*-methyl-D-glucamine$^+$ and 0.5 mM Mg^{2+} (divalent) reduced Zn uptake to a similar extent (~50%), indicating a relatively nonspecific competition for anionic sites on the gill. Ca^{2+} was an exception and was more potent at reducing Zn uptake, likely because only Ca^{2+} would also compete for absorption. Although Na^+ and Mg^{2+} were able to markedly reduce Zn uptake, they had no effect on Zn toxicity (measured with 96-h LC_{50} tests), a result paralleled by their inability to restore Ca^{2+} uptake that was inhibited by Zn. In contrast, Ca^{2+} reduced Zn toxicity and restored Ca^{2+} uptake. These results partially dissociate Zn uptake from Zn toxicity, implicate disturbed Ca^{2+} uptake as the toxic mechanism, and have profound implications for water quality criteria where Ca^{2+} and Mg^{2+} (the two "hardness" cations) are traditionally considered to be equally protective.

Résumé : Les effets des cations aqueux sur l'absorption du ^{65}Zn, la toxicité du Zn et les relations avec l'absorption du calcium ont été étudiés chez des truites arc-en-ciel juvéniles en eau douce. L'absorption par tout l'organisme du Zn présent dans l'eau ([Zn] = 100 $\mu g \cdot L^{-1}$ = 1,5 μM a été fortement réduite par divers cations. Cette réduction était directement liée à la concentration des charges positives, indépendamment de l'ion qui portait ces charges. Par conséquent, 1,0 mM de Na^+, K^+, NH_4^+ ou de *N*-méthyl-D-glucamine$^+$ et 0,5 mM Mg^{2+} (divalent) réduisaient l'absorption du Zn d'une façon semblable (~50%), ce qui montre une concurrence relativement non spécifique pour les sites anioniques des branchies. Le Ca^{2+} faisait cependant exception et provoquait une plus grande réduction de l'absorption du Zn, sans doute parce que le Ca^{2+} exerce aussi une concurrence pour l'absorption. Bien que le NA^+ et le Mg^{2+} pouvaient réduire l'absorption du Zn de façon marquée, ils n'avaient aucun effet sur la toxicité du Zn (déterminée par la CL_{50} 96-h), un effet qui correspond à leur incapacité de rétablir l'absorption du Ca^{2+} qui avait été inhibée par le Zn. Au contraire, le Ca^{2+} réduisait la toxicité du Zn et rétablissait l'absorption du Ca^{2+}. Ces résultats dissocient, du moins en partie, l'absorption et la toxicité du Zn et font supposer une perturbation de l'absorption du Ca^{2+} en tant que mécanisme de toxicité. Cela a d'importantes incidences sur l'établissement des critères de qualité de l'eau pour lequel on a toujours supposé que le Ca^{2+} et le Mg^{2+} (deux cations de la « dureté ») avaient un rôle protecteur semblable.

[Traduit par la Rédaction]

Introduction

Zinc is an essential micronutrient that can be absorbed by fish via the gill from the aquatic environment as well as from the diet (Spry et al. 1988). The pathway of branchial Zn uptake (as well as other cations) begins with the adsorption of the cation to a negatively charged site on the gill surface (Pagenkopf 1983), followed by absorption into the gill cell (Hogstrand et al. 1996). Different cations can compete for these negative sites on the gill surface. Handy and Eddy (1991) showed that Na^+ adsorption (and consequently absorption into the gill) was reduced by competition from other waterborne cations such as H^+ and Ca^{2+} for binding sites.

If waterborne Zn concentrations become high enough, too much adsorption/absorption may ultimately lead to toxic effects. Zn specifically disrupts Ca^{2+} uptake at the gill, which can lead to hypocalcemia and even death of the fish (Spry and Wood 1985; Hogstrand et al. 1995). Zn toxicity is not only dependent on Zn concentration but also on the presence of other ions in the water. In water quality regulations (e.g., U.S. Environmental Protection Agency 1980), water hardness (the sum of Mg^{2+} and Ca^{2+} expressed as milligrams of $CaCO_3$ per litre) is the only component of water quality that is taken into account as a modifying agent for Zn toxicity. For example, a decrease in water hardness of about 10 times was shown to increase Zn toxicity to juvenile rainbow trout, *Oncorhynchus mykiss*, by 10 times (Bradley and Sprague 1985). Similarly, Alsop et al. (1999) reported that a sixfold

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Table 1. Water composition of hard water (Hamilton tap water from nearshore Lake Ontario) and artificial soft water of the Zn and Ca^{2+} uptake experiments and LC_{50} trials.

	Hard water	Softwater Zn uptake	Softwater Zn LC_{50}/Ca^{2+} uptake
Ca^{2+} (mM)	1.0	0.02	0.06
Mg^{2+} (mM)	0.2	0.01	0.02
Na^{+} (mM)	0.6	0.04	0.05
K^{+} (mM)	0.05	nd	0.01
Cl^{-} (mM)	0.7	0.04	0.05
Hardness (as mg $CaCO_3 \cdot L^{-1}$)	120	5	10
pH	8.0	5.6	5.8

Note: nd, not detectable.

reduction in hardness increased toxicity about six times. The toxic effects of Zn, like most metals, are therefore of particular concern in ion-poor water.

Other ions may also compete with Zn binding to the gill, and any ions that impede the initial adsorption of Zn to the gill might be expected to reduce its toxicity (Simkiss and Taylor 1989). For example, Pagenkopf (1983) predicted that H^{+} would compete with cationic metals for binding sites on the gill, leading to lower toxicity at sublethal low pH. While data were not available for Zn at the time, the protective effect of sublethal low pH was subsequently shown experimentally by both Bradley and Sprague (1985) and Cusimano et al. (1986). In contrast, Pagenkopf (1983) discounted the protective effect of other monovalents such as Na^{+} and K^{+} because of their "less extensive" interactions with the gill.

One aim of the present study on freshwater rainbow trout acclimated to soft water was to look at the effect of a range of different cations on radiolabelled Zn (^{65}Zn) uptake and the relative potencies of their inhibition. The use of the radioisotope ^{65}Zn was required to measure Zn accumulation (Galvez et al. 1998; Hogstrand et al. 1998) due to the high levels of Zn already present in fish tissues, which reflect its role as a micronutrient. We predicted that Ca^{2+} would have the largest influence on Zn uptake due to the fact that not only would Ca^{2+} compete with Zn for binding sites on the gill surface, but it would also compete for uptake. Recent evidence indicates that Ca^{2+} and Zn share the same apical transport channel in the gill (reviewed by Hogstrand and Wood 1996). Another goal of this study was to determine whether reductions in Zn uptake by various cations translate into a protective effect against Zn toxicity. Of particular interest was the relative effectiveness of Ca^{2+} and Mg^{2+}, the two major components of water "hardness," in influencing both Zn uptake and Zn toxicity. Finally, since inhibition of branchial Ca^{2+} uptake may be the mechanism of Zn-induced mortality, we examined whether protection against acute Zn toxicity (i.e., increased 96-h LC_{50}) by a particular cation was associated with a restoration of Ca^{2+} uptake.

Materials and methods

Fish care

Juvenile rainbow trout (3–10 g) were obtained from Rainbow Springs Trout Hatchery (Thamesford, Ont.) and initially held for 1 week in aerated 500-L tanks supplied with dechlorinated Hamilton tap water at 3 $L \cdot min^{-1}$ (Table 1). The fish were then slowly acclimated to "soft water" over the next 2 weeks by mixing hard water with increasing amounts of ion-reduced water (produced by reverse osmosis; Anderson Water Systems, Dundas, Ont.) until the desired water chemistry was reached (Table 1). The fish were allowed to acclimate to this soft water for at least 1 month before experimentation. Soft water was favored for Zn uptake experiments because >99% of waterborne Zn would be in the free ionic form (Zn^{2+}) at the low pH and alkalinity characteristic of this water, as predicted with the aquatic geochemical program MINEQL+ (Schecher and McAvoy 1994). Trout were fed to satiation three times per week with commercial trout feed (fish food composition (partial analysis only): crude protein (minimum), 52%; crude fat (minimum), 17%; crude fibre (maximum), 2.5%; water, 12%; Ca^{2+}, 1.4%; Na^{+}, 0.4%; Zn (measured), 0.02% (173 $\mu g \cdot g^{-1}$)). Water temperature was 10 ± 1°C during the experiments.

Zn uptake

Twenty-litre buckets served as ^{65}Zn flux units and were lined with polypropylene bags. The buckets were filled with 14 L of soft water (ionic composition given in Table 1) and placed in a wet table surrounded by flowing water for temperature regulation. A stock Zn solution was added to each flux unit (as $ZnSO_4 \cdot 7H_2O$; Anachemia) to bring each one to 100 μg $Zn \cdot L^{-1}$ (1.53 μM). In addition, 10 μCi (0.37 MBq) of ^{65}Zn (as $ZnCl_2$, specific activity = 1.97 $mCi \cdot mg^{-1}$; NEN Life Science Products, Boston, Mass.) was added to each flux unit, an amount that had negligible influence on the total Zn concentration of the water. Each trial was run with a control and with the addition of 0.5, 1.0, and 2.0 mM of a test salt, unless otherwise noted. Once the flux units were prepared and equipped with gentle aeration, 28 fish were removed from their holding tank and divided evenly between the four flux units (control, 0.5, 1.0, and 2.0 mM test salt; $N = 7$). Flux experiments lasted 7 h, with triplicate 5-mL water samples taken just before the termination of the flux. At the end of the flux period, the fish were removed and rinsed for 1 min in a bath containing a lethal amount of anesthetic (0.5 g MS 222·L^{-1}) and 40 mg $Zn \cdot L^{-1}$ (as $ZnSO_4 \cdot 7H_2O$) to displace any surface-bound ^{65}Zn. This protocol was chosen based on the results of Spry and Wood (1989) who showed that there is a rapid initial adsorption of ^{65}Zn to sites on the surface of the fish, followed by linear uptake thereafter. The 7-h flux period ensured that most of the uptake was measured over the linear period, and the 40 $mg \cdot L^{-1}$ cold Zn rinse served to minimize (by displacement) the initial adsorption component. After rinsing, trout were blotted dry and weighed to the nearest 0.01 g. Fish were assayed for ^{65}Zn activity in a gamma counter (MINAXI Auto-Gamma 5000 Series, Canberra-Packard). Water samples were similarly assayed for ^{65}Zn activity as well as for total Zn and the concentration of the cation being tested (Na^{+}, K^{+}, Mg^{2+}, or Ca^{2+}) by atomic absorption spectrophotometry. NH_4^{+} concentrations were determined by the method of Verdouw et al. (1978).

The following salts were tested: NaCl, $NaNO_3$, KCl, NH_4Cl, $MgCl_2$, and $CaCl_2$ (BDH Chemicals). There was no indication of ammonia toxicity during the 7-h flux experiment (i.e., there were no mortalities and swimming behavior appeared normal). *N*-Methyl-D-glucamine chloride (Sigma) was also tested by first preparing a stock solution, which was then titrated to pH 7.0 with concentrated HCl. $MgCl_2$ and $CaCl_2$ were tested solely at 0.5 mM against 0.5 mM NaCl and a control. In addition, the effects of pH on ^{65}Zn uptake were investigated. Two tanks were set up as described above, a control tank (pH 5.6) and a tank at pH 3.7 (addition of ~0.2 mM H^{+}). pH levels were achieved by the addition of concentrated HCl to the water, and levels were constantly monitored during the flux and adjusted if needed.

To determine if acclimation to the test salts had an effect on their inhibition of Zn uptake, 20 fish were acclimated to soft water plus 1 mM Na^{+} (as NaCl) and 20 fish to soft water plus 1 mM Ca^{2+}

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(as $CaCl_2$) for 3 weeks. During acclimation, trout were kept in 30-L tanks and 90% of the water was replaced every 2 days. Fish were fed to satiation as usual. Flux tests were then performed in 1 mM NaCl and 1 mM $CaCl_2$ with acclimated and nonacclimated fish against a softwater control with nonacclimated fish.

Finally, an experiment was performed to examine the distribution of radiolabelled Zn accumulation during the flux tests in the absence (control) and presence of 1.0 mM Na^+. The flux test was performed as described above. At the end of 7 h, fish were rinsed and lethally anaesthetized as above, and then, blood was collected in heparinized capillary tubes by caudal severance, the gills were removed and blotted dry, and a piece of skin was removed from the side of the fish underneath the dorsal fin. Any musculature adhering to the skin was scraped off. The height of the blood collected in capillary tubes was measured in millimetres (internal radius of tubes = 1.15 mm) to determine its volume, and the other tissues and remaining carcass were weighed to the nearest 0.001 g. All samples were assayed for ^{65}Zn. For calculations of partitioning, the blood was assumed to account for 5% of the body mass (Olson 1992), the skin was assumed to weigh 0.014 $g \cdot cm^{-2}$, and the total skin was assumed to equal 1.36 $cm^2 \cdot g\ fish^{-1}$ based on measurements carried out by M. Groselli (Department of Biology, McMaster University, Hamilton, Ont., personal communication).

Expressed concentrations of Zn and cations are nominal, but were always within 10% of the desired concentrations.

Toxicity testing

To test whether inhibition of Zn uptake had an effect on acute Zn-induced mortality, 96-h LC_{50} tests were conducted. The four test conditions were soft water (Table 1), soft water plus 2.0 mM Na^+, soft water plus 1.0 mM Mg^{2+}, and soft water plus 1.0 mM Ca^{2+}.

Forty trout were removed and divided into five 18-L tanks (eight fish per tank, with each tank receiving 200 mL soft $water \cdot min^{-1}$) and allowed to settle for 1 h. Tanks were then randomly assigned to one of five Zn concentrations including a control group (0–350 µg $Zn \cdot L^{-1}$, 0–3000 µg $Zn \cdot L^{-1}$ for the Ca^{2+} trial). To begin the LC_{50}, flow of toxicant from a Mariotte bottle was started into a head tank where it mixed with inflowing fresh water by vigorous aeration. The toxicant consisted of $ZnSO_4 \cdot 7H_2O$, with the appropriate concentration of cationic salt if required, dissolved in deionized water acidified with 0.2 mL concentrated $HNO_3 \cdot L^{-1}$ (trace metal analysis grade; BDH Chemicals). At the same time, Zn and the appropriate cation were added to each tank (apart from the control tanks) to immediately bring them up to the desired Zn concentrations. Mortalities were recorded over 96 h. Water samples were taken daily and acidified for later analysis. The 96-h LC_{50}s ± 95% confidence limits (CL) were calculated by log-probit analysis of mortality versus measured waterborne Zn concentration (Finney 1971).

Ca^{2+} uptake

The effect of various ions on the ability of Zn to disrupt Ca^{2+} uptake was investigated in an attempt to determine their protective mechanism. Six flux units were set up as for the ^{65}Zn fluxes above. The tanks were designated as (*i*) control soft water, pH = 5.8, (*ii*) soft water plus 100 µg $Zn \cdot L^{-1}$, pH = 5.8, (*iii*) soft water plus 100 µg $Zn \cdot L^{-1}$ with pH adjusted to 4.0 with HCl, (*iv*) soft water plus 100 µg $Zn \cdot L^{-1}$ plus 2 mM Na^+, (*v*) soft water plus 100 µg $Zn \cdot L^{-1}$ plus 1 mM Mg^{2+}, and (*vi*) soft water plus 100 µg $Zn \cdot L^{-1}$ plus 1 mM Ca^{2+}, all as chloride salts. Fifteen microcuries of ^{45}Ca (as $CaCl_2$; NEN Life Science Products) was added to each flux unit and the fish (N = 8) were exposed for 7 h. In a separate experiment, the time course of the effect of low pH on Zn-induced inhibition of Ca^{2+} uptake was investigated. Trout were exposed to ^{45}Ca in soft water, soft water plus 100 µg $Zn \cdot L^{-1}$, and soft water plus 100 µg $Zn \cdot L^{-1}$ at pH 4.1, all for 5 h. Sixteen fish were also held in 100 µg $Zn \cdot L^{-1}$ and 100 µg $Zn \cdot L^{-1}$ at pH 4.1 for 8 and 30 h without ^{45}Ca and then exposed to ^{45}Ca for 5 h.

At the termination of the ^{45}Ca fluxes, water samples were taken and fish were removed and rinsed for 1 min in 0.5 g MS $222 \cdot L^{-1}$ and 5 mM $CaCl_2$ to displace any surface-bound ^{45}Ca. Fish were weighed and digested in a vial with five times their weight of tissue solubilizer (TS-2; Research Products International, Mount Prospect, Ill.). Digestion was accelerated by heating to 55°C for 36 h. Triplicate 0.5-mL samples of the digest media and water were assayed for ^{45}Ca by scintillation counting using an LKB 1217 Rackbeta (Hamilton, Ont.). Differences in counting efficiency between digest media and water were corrected by internal standardization.

Calculations and statistics

The appearance of ^{65}Zn from the water into the fish was calculated from the ^{65}Zn activity of the whole body and the specific activity of Zn in the water. The mean specific activity (SA) of Zn in the water over the flux period was calculated as

$$(1) \qquad SA = (cpm \cdot mL^{-1})/[Zn]$$

where cpm are the gamma counts per minute and [Zn] is the concentration of Zn (micrograms per millilitre). In practice, Zn total concentrations and specific activities underwent negligible change during these tests. Total Zn appearance in the body was then calculated as

$$(2) \qquad \text{Total Zn appearance} = (\text{cpm/body weight})(1/SA)$$

where total Zn appearance was in micrograms of Zn per gram of body, which was then divided by 7 h (the duration of the exposure) to yield a final uptake rate in micrograms of Zn per gram of body per hour. Ca^{2+} uptake rates using ^{45}Ca were calculated in an analogous fashion.

Data have been expressed as means ± SE (N) except for the 96-h LC_{50}s where means ± 95% CL have been reported. For the ^{65}Zn and ^{45}Ca uptake experiments, significant differences from the control uptake rate were tested with a one-way analysis of variance (ANOVA) followed by Tukey's HSD test for multiple comparisons to determine significant differences among treatments. LC_{50}s were compared by means of the Bonferroni adjustment to the independent two-tailed Student t test. The limit of significance was 5, 1, and 0.1% (indicated on the graphs as one, two, and three asterisks, respectively).

Results

Zn uptake

Rainbow trout exposed to 100 µg $Zn \cdot L^{-1}$ had a control whole-body uptake rate of 0.033 µg $Zn \cdot g\ body^{-1} \cdot h^{-1}$ (Fig. 1A). Na^+, as NaCl, at 0.5, 1.0, and 2.0 mM reduced the control whole-body Zn uptake by 26, 48, and 87%, respectively (Fig. 1A). As well, Na^+, as $NaNO_3$, at 0.5, 1.0, and 2.0 mM also reduced Zn uptake by 33, 67, and 86%, respectively, showing that the effect was independent of the anion (Fig. 1B). The chloride salts of K^+ reduced Zn uptake by 42, 75, and 93% (Fig. 2A), those of NH_4^+ by 53, 52, and 80% (Fig. 2B), and those of *N*-methyl-D-glucamine by 37, 52, and 37%, respectively (Fig. 2C), all at 0.5, 1.0, and 2.0 mM concentrations, very similar effects to those of Na^+. *N*-Methyl-D-glucamine was tested because it is generally considered to be impermeable across biological membranes.

The addition of 0.5 mM Na^+ (monovalent), 0.5 mM Mg^{2+} (divalent), and 0.5 mM Ca^{2+} (divalent), all as chloride salts, decreased Zn uptake by 20, 53, and 85%, respectively, from a control uptake rate of 0.039 µg $Zn \cdot g\ body^{-1} \cdot h^{-1}$ (Fig. 3). The effect of 0.5 mM Ca^{2+} was significantly greater than

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Fig. 1. Effects of (A) NaCl and (B) $NaNO_3$ on whole-body Zn uptake measured with ^{65}Zn as a tracer in juvenile rainbow trout. The total Zn concentration of the water was 100 $\mu g \cdot L^{-1}$. Values are expressed as means ± SE; $N = 7$. One asterisk denotes a significant decrease in Zn uptake from the control treatment as determined by a one-way ANOVA followed by Tukey's HSD test ($p < 0.050$, two asterisks denote $p < 0.01$, and three asterisks denote $p < 0.001$).

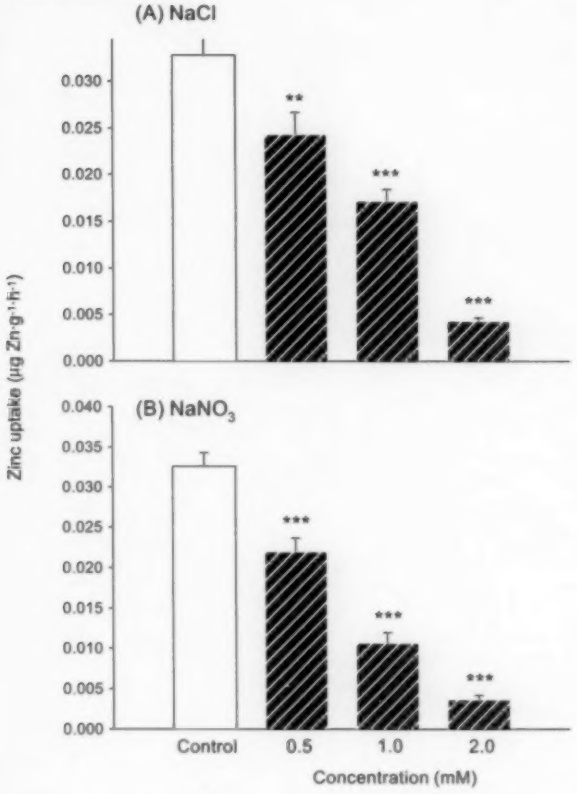
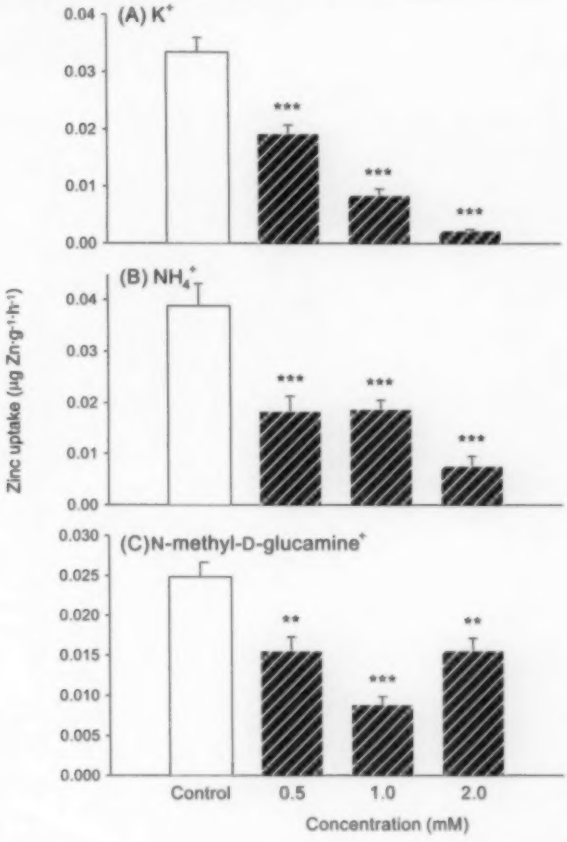


Fig. 2. Effects of (A) KCl, (B) NH_4Cl, and (C) *N*-methyl-D-glucamine chloride (with a single positive charge) on whole-body Zn uptake. Refer to Fig. 1 caption for more details.



that of 0.5 mM Mg^{2+}, which in turn was significantly greater than that of 0.5 mM Na^+.

When pH was decreased by about 2 pH units from 5.6 to 3.7 (a 100-fold increase in H^+ concentration using HCl), Zn uptake was significantly decreased by 74% from 0.007 ± 0.00021 to 0.0018 ± 0.00012 μg Zn·g body^{-1}·h^{-1} ($p < 0.001$). As pH 3.7 represents the addition of about 0.2 mM H^+, protons were clearly more potent than the other cations tested in antagonizing Zn uptake.

Acute versus chronic effects of cation exposure were evaluated. Whether trout were acclimated to soft water plus 1.0 mM Na^+ for 3 weeks prior to testing or were acutely exposed to 1.0 mM Na^+ only at the time of the experiment, the presence of 1.0 mM Na^+ in the water had the same effect in decreasing Zn uptake (Fig. 4). This was also the case for Ca^{2+}, where acclimation to 1.0 mM Ca^{2+} had the same effect on reducing the uptake rate of Zn as did acute exposure to 1.0 mM Ca^{2+}, in comparison with control trout (Fig. 4).

In the test to determine the internal distribution of radiolabelled Zn taken up from the water over the 7-h period, the majority (71%) was in the carcass in the control treatment (Fig. 5B). The gills, which accounted for only 2.0% of the body weight, held 17% of the Zn, the blood, which accounted for 5% of the body weight (Olson 1992) held 10% of the Zn, and the skin, which accounted for 0.7% of the body weight, held only 2.5% of the Zn (Fig. 5B). In this experiment, the presence of 1 mM NaCl reduced overall Zn uptake by 30% (Fig. 5A). By far, the largest absolute reduction was in the carcass (29%), with most of the remaining reduction occurring in the gills. There was no significant reduction in the blood or skin components. This result shows that the presence of the competing cation inhibits mainly internal Zn accumulation and is not just acting by displacing Zn from the body surface.

Toxicity testing

The addition of 2 mM NaCl or 1 mM $MgCl_2$ to the water had no effect on acute Zn toxicity, as evaluated by 96-h LC_{50} tests (Fig. 6A). In contrast, the addition of 1 mM $CaCl_2$ greatly reduced Zn toxicity, as indicated by the 18-fold increase in the LC_{50} from a control value of 103 $\mu g \cdot L^{-1}$ to 1800 $\mu g \cdot L^{-1}$ (Fig. 6A).

Ca^{2+} uptake

Exposure to 100 μg Zn·L^{-1} in control soft water signifi-

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Fig. 3. Effects of 0.5 mM NaCl, $MgCl_2$, and $CaCl_2$ on whole-body Zn uptake. Refer to Fig. 1 caption for more details.

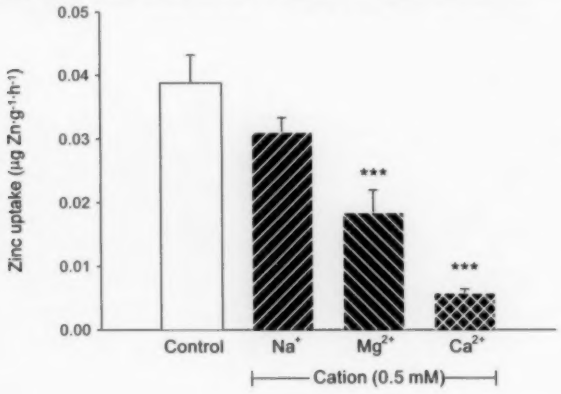
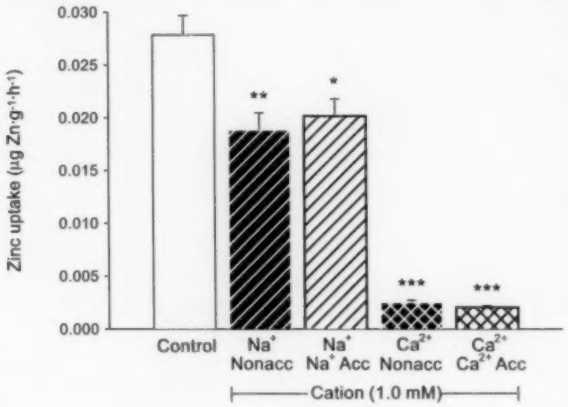


Fig. 4. Effects of 1 mM NaCl and 1 mM $CaCl_2$ on whole-body Zn uptake in 1 mM NaCl and 1 mM $CaCl_2$ acclimated (3 weeks) and nonacclimated rainbow trout. Refer to Fig. 1 caption for more details.



cantly reduced Ca^{2+} uptake by 87% (Fig. 6B). The addition of 2 mM NaCl, 1 mM $MgCl_2$, or 0.2 mM HCl (pH 4.0; data not shown) did not diminish the effect of Zn on Ca^{2+} uptake over 7 h. However, the addition of 1 mM $CaCl_2$ significantly increased Ca^{2+} uptake back to 54% of the control rate, a value that was not significantly different from the control (Fig. 6B). In addition, short-term (0–5 h) or long-term (up to 35 h) exposure to Zn with the addition of 0.2 mM HCl (pH 4.1) did not restore Ca^{2+} uptake (Table 2).

Discussion

Zn uptake

Whole-body Zn uptake was reduced in the presence of a variety of positively charged waterborne ions. The magnitude of the reduction was directly related to the concentration of positive charges in the water (e.g., equivalents), regardless of which element or compound carried that charge (except for Ca^{2+} and H^+, as discussed below). Thus, on a molar basis, divalent Mg^{2+} was about twice as potent as the monovalent cations. We interpret the slight differences among different monovalent cations on inhibition of Zn uptake as variation of the effect as opposed to real differences in inhibition potency (except for Ca^{2+} and H^+, as discussed below). For example, 0.5 mM Na^+ decreased Zn uptake from control treatments by 26% (Fig. 1A), 33% (Fig. 1B), and 20% (Fig. 3) in three different trials. Free Zn^{2+} activity would not have changed significantly with the addition of each salt as a result of ionic strength effects as predicted by the Debye–Hückel equation for the range of concentrations tested in the present study (up to 2 mM). Significant reductions in activity would occur when salts exceed about 10 mM (Stumm and Morgan 1996).

Significant reductions of Zn uptake were observed at environmentally realistic freshwater concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} (see Table 1 for typical freshwater Lake Ontario ionic composition, "hard water"). The Zn exposure concentrations in the uptake studies were also in the range of environmental relevance. While normal Zn levels in clean freshwaters are only a few micrograms per litre or less, concentrations of 50 $\mu g \cdot L^{-1}$ are found in industrialized areas. Maximum Zn concentrations in natural surface waters are reported to range from 130 to 1170 $\mu g \cdot L^{-1}$ in different areas of Canada (Canadian Council of Ministers of the Environment 1995). The Zn concentration used in the uptake studies was the same as our measured 96-h LC_{50} (~100 $\mu g \cdot L^{-1}$) (Fig. 6A). The Zn uptake rates in the present study were comparable with those reported by Hogstrand et al. (1998) at 100 μg $Zn \cdot L^{-1}$ in juvenile rainbow trout in very soft water (Ca^{2+} free). They were also comparable with uptake rates at total Zn concentrations near the LC_{50} (~900 $\mu g \cdot L^{-1}$, Alsop et al. 1999) in Hamilton hard water (Hogstrand et al. 1998). Nevertheless, the Zn uptake rate did vary over the course of the present study from a high of 0.039 $\mu g \cdot g^{-1} \cdot h^{-1}$ (Figs. 2B and 3) to a low of 0.007 $\mu g \cdot g^{-1} \cdot h^{-1}$ (Zn uptake and pH trial) in different control series. The lower rates occurred towards the end of the study when the fish were larger. Notably, Wagner et al. (1985) found that branchial Ca^{2+} uptake cycled between periods of low uptake and high uptake in rainbow trout. The difference in Ca^{2+} uptake rates varied on the order of fivefold, while the duration of the cycles varied from 7 to 17 days; hormonal changes were thought to be involved, and Zn uptake may follow a similar trend. However, nothing is known about possible corresponding trends in Zn efflux, an important topic for future research.

Ca^{2+} had a much greater effect inhibiting Zn uptake than the other divalent cation tested, Mg^{2+} (Fig. 3). This is likely because Zn^{2+} and Ca^{2+} would not only compete on the gill surface for binding sites (adsorption) but would also compete for absorption, since they share the same apical transport channel (Hogstrand et al. 1996). Zn and Ca^{2+} may also interact with each other inside the gill cell, where, for example, Zn has been shown to decrease Ca^{2+} uptake by both the endoplasmic reticulum and mitochondria (Verbost et al. 1996). Zn is also a potent inhibitor of the basolateral CaATPase transporter in the trout gill (Hogstrand et al. 1996). Increasing waterborne Ca^{2+} may also elicit stanniocalcin release into the blood, which would cause closure of

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Fig. 5. (A) Effect of 1 mM NaCl on whole-body Zn uptake and (B) partitioning of ^{65}Zn into the gills, skin, blood, and remaining carcass of rainbow trout. Uptake was significantly reduced in the gills and remaining carcass exposed to 1 mM Na^+. The 1 mM Na^+ did not have an effect on the amount of ^{65}Zn bound to the exterior of the fish. $N = 8$. Refer to Fig. 1 caption for more details.

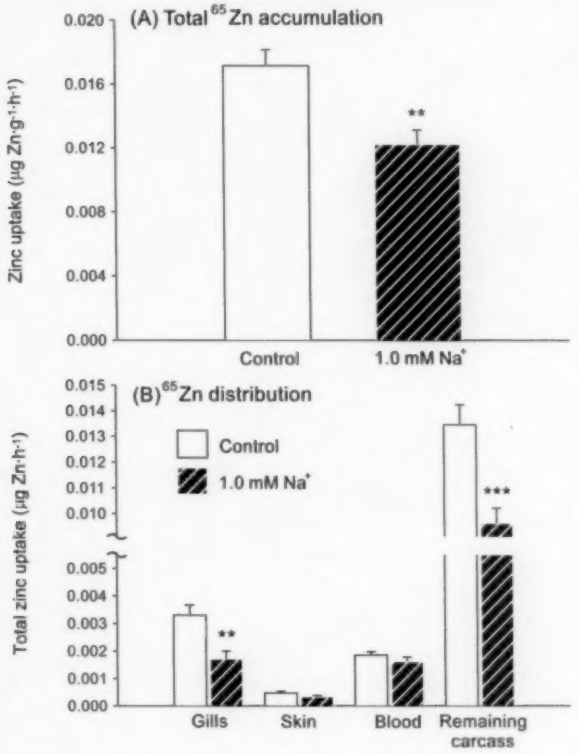


Table 2. Whole-body Ca^{2+} uptake (μg $Ca \cdot g^{-1} \cdot h^{-1}$) in control soft water and during exposure to 100 μg $Zn \cdot L^{-1}$ at pH 5.8 (control pH) or 100 μg $Zn \cdot L^{-1}$ at pH 4.1 over 35 h.

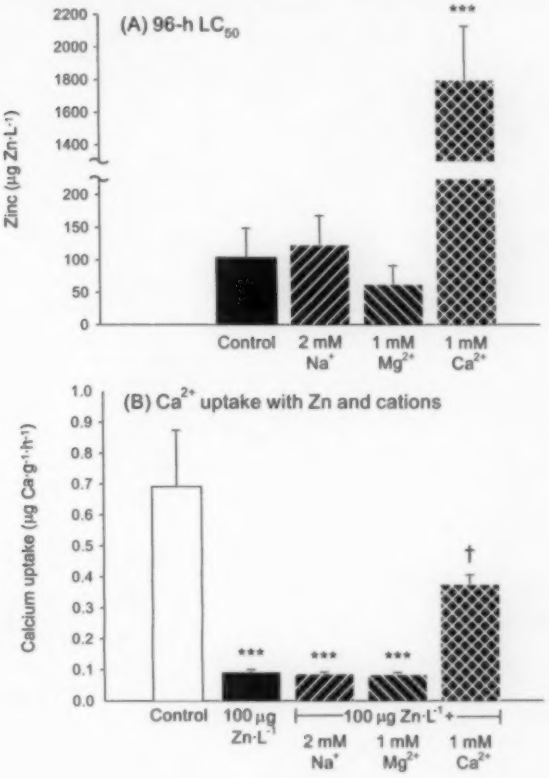
Time (h)	Control	100 μg $Zn \cdot L^{-1}$	100 μg $Zn \cdot L^{-1}$ at pH 4.1
−5 to 0	3.05±0.24		
0–5		0.40±0.17***	0.22±0.01***
8–13		0.21±0.01***	0.18±0.01***
30–35		0.20±0.01***	0.17±0.002***

Note: For the 8- to 13-h and 30- to 35-h ^{45}Ca exposures, rainbow trout were initially held in identical Zn and pH waters without ^{45}Ca until they were used in the ^{45}Ca flux. Values are expressed as means ± SE; $N = 8$. One asterisk denotes a significant decrease in Ca^{2+} uptake from the control treatment as determined by a one-way ANOVA followed by Tukey's HSD test ($p < 0.05$), two asterisks denote $p < 0.01$, and three asterisks denote $p < 0.001$.

the apical Ca^{2+}/Zn^{2+} channels in the gill and decrease both Zn and Ca^{2+} uptake (Wendelaar Bonga and Pang 1991).

NH_4^+ had the same effect in reducing Zn uptake as the other monovalent cations that were tested (Fig. 2B). This indicates that not only can cations from the water affect Zn uptake, but cations that are excreted by the fish at the gills

Fig. 6. (A) 96-h LC_{50} values in control water alone (no additions except for Zn) and with the addition of 2 mM NaCl, 1 mM $MgCl_2$, or 1 mM $CaCl_2$. Values are expressed as means ± the average of the upper and lower 95% CL. Refer to Fig. 1 caption for more details. (B) Ca^{2+} uptake in control soft water alone (no additions) and with the addition of 100 μg $Zn \cdot L^{-1}$ or either 2 mM NaCl, 1 mM $MgCl_2$, or 1 mM $CaCl_2$ in addition to 100 μg $Zn \cdot L^{-1}$. Values are expressed as means ± SE; $N = 8$. One asterisk denotes a significant decrease in Ca^{2+} uptake from the control treatment as determined by a one-way ANOVA followed by Tukey's HSD test ($p < 0.05$), two asterisks denote $p < 0.01$, and three asterisks denote $p < 0.001$. A dagger indicates a significant increase in Ca^{2+} uptake over the 100 μg $Zn \cdot L^{-1}$ treatment ($p < 0.001$). Note that the 1 mM $CaCl_2$ mean was not significantly different ($p > 0.05$) from the control mean.



may also affect uptake. Zn uptake may therefore be reduced during times of high ammonia excretion, which can occur, for example, after feeding (Kaushik 1980; Beamish and Thomas 1984; Alsop and Wood 1997).

Cationic competition most probably occurs at the apical surface of the gill. *N*-Methyl-D-glucamine$^+$ is a molecule with a single positive charge that is generally considered to be impermeant to biological membranes (e.g., Song and Chueh 1996), so any effects from the presence of that molecule can only occur in the water or at the gill surface. Therefore, the reduction in Zn absorption (Fig. 2C) was due to decreased Zn adsorption to the gill surface due to competition with the charge carried by *N*-methyl-D-glucamine$^+$.

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Decreasing the pH to 3.7 (adding 0.2 mM H^+) reduced Zn uptake to a greater extent than did the other monovalent ions tested. With the drop in pH over this range, there would be no change in Zn speciation as determined by the aquatic geochemical program MINEQL+ (Schecher and McAvoy 1994), which predicts >99% of the Zn to exist in the "free" form (Zn^{2+}) in both control and decreased-pH test waters. H^+ is not known to be transported through the Ca^{2+}/Zn^{2+} channel, and therefore, direct competition should not be occurring there. Handy and Eddy (1991) found an increased potency of H^+ in comparison with Ca^{2+} in reducing Na^+ adsorption and absorption in rainbow trout. They attributed this to the increased mobility of H^+ due to its decreased "charge density" in comparison with Ca^{2+}. An alternative mechanism may be direct acid damage to the Zn^{2+}/Ca^{2+} transport system or an indirect effect, such as stimulation of mucus secretion by low pH (McDonald 1983). Mucus production may slough Zn off before it can be taken up into the gill, and once the mucus is sloughed off, it may even bind Zn in the water, keeping it off the gill.

Pagenkopf (1983) hypothesized that H^+ would compete for the negative sites on the gill surface, forming Lewis acid–base complexes, unlike Na^+ and K^+. Playle et al. (1993) showed that H^+ had a high affinity for Cd-binding sites on the gills, with log *K* stability constants for Cd, H^+, and Ca^{2+} of 8.6, 6.7, and 5.0, respectively. Like Zn, Cd disrupts Ca^{2+} homeostasis (Verbost et al. 1989). Galvez et al. (1998) estimated the log *K* value for Zn^{2+} and trout gills to be about 5.5. Richards and Playle (1998) found a similar pattern of potency in their study of Co binding to rainbow trout gills, and Co is also known to interfere with Ca^{2+} uptake (Comhaire et al. 1994). Richards and Playle (1998) reported that H^+ was the most potent cation reducing Co accumulation in the gills, followed by Ca^{2+} and then Na^+.

Toxicity testing

From the present study, it appears that any cation may possess the capability of reducing the uptake of Zn at trout gills. Simkiss and Taylor (1989) stated that anything that obstructs the initial adsorption of a metal will reduce its toxicity. The present study showed that this is not always the case. Although Na^+ and Mg^{2+} were able to reduce Zn uptake, presumably through decreased adsorption to the gill (Pagenkopf 1983) (Figs. 1 and 3), they did not ameliorate Zn toxicity (Fig. 6A). In contrast, Ca^{2+} strongly reduced both Zn uptake and Zn toxicity (Figs. 3 and 6A).

One explanation may be that Na^+ and Mg^{2+} may indeed reduce Zn uptake by decreasing adsorption to Zn uptake sites, but these may not be the sites related to Zn toxicity. Ca^{2+} may reduce adsorption to both nontoxic Zn uptake sites and to a separate population of toxic Zn binding sites. Another explanation may be that cations such as Na^+ are reducing nontoxic Zn uptake, while Ca^{2+} reduces both toxic and nontoxic Zn uptake (as opposed to binding). A third possibility relates to effects on Ca^{2+} uptake. Zn specifically induces hypocalcemia by inhibiting branchial Ca^{2+} uptake (Spry and Wood 1985; Hogstrand et al. 1995), and increased waterborne Ca^{2+} may simply reduce the symptoms of Zn toxicity by preventing this effect. Indeed, we found that 2 mM Na^+ and 1 mM Mg^{2+} did not restore Zn-induced Ca^{2+} uptake inhibition (Fig. 6B), nor did they reduce Zn-induced mortality (Fig. 6A). However, 1 mM Ca^{2+} was able to restore branchial Ca^{2+} uptake that had been inhibited by 100 μg $Zn{\cdot}L^{-1}$ (Fig. 6B), a Zn concentration that was equivalent to the 96-h LC_{50} in control soft water (Fig. 6A). In addition, 1 mM Ca^{2+} simultaneously increased the LC_{50} to 1800 μg $Zn{\cdot}L^{-1}$, a Zn concentration that was probably high enough to inhibit branchial Ca^{2+} uptake again. These results partially dissociate Zn uptake from acute Zn toxicity and reinforce the view that Zn-induced inhibition of Ca^{2+} uptake is the direct cause of toxicity.

Both Bradley and Sprague (1985) and Cusimano et al. (1986) observed a protective effect of decreased pH (increased H^+) at nontoxic levels (pH ≥ 4.7) against waterborne Zn toxicity. Cusimano et al. (1986) speculated that H^+ interference with metal uptake at the gill was the primary mechanism that decreased metal toxicity (interestingly, they found that H^+ also protected against Cu and Cd toxicity). In the present study, however, a higher [H^+] (pH 4.0, close to the toxic range) did not restore Ca^{2+} uptake over 7 h of exposure. Hobe et al. (1984) found H^+ (pH 4.0–4.2) alone initially reduced Ca^{2+} uptake in rainbow trout by two thirds from 0 to 12 h, but Ca^{2+} uptake was fully restored by 18–24 h. We suspected that if pH was protective, it may require a longer period of time. However, we found no effect of H^+ (pH 4.1) on restoring Zn-induced Ca^{2+} uptake inhibition again over short-term (0–5 h) or long-term (up to 35 h) exposures (Table 2). If H^+ is truly protective against Zn toxicity, it may protect via a different mechanism than does Ca^{2+}.

With respect to Zn, there is also a general acceptance of the principle that acute toxicity is related to the levels of waterborne Ca^{2+} and Mg^{2+}, which is termed "hardness" (Canadian Council of Ministers of the Environment 1995). For example, the U.S. Environmental Protection Agency (1980) employs an equation based on hardness (milligrams of $CaCO_3$ per litre) to calculate a numerical limit of total allowable waterborne Zn:

$$(3) \qquad [Zn] = e^{(0.83[\ln(\text{hardness})]+1.95)}\ \mu g{\cdot}L^{-1}.$$

The present study has shown that while Ca^{2+} protects against acute Zn toxicity, Mg^{2+} does not. Therefore, eq. 3 would overestimate safe Zn levels in waters where a significant percentage of the total hardness was contributed by Mg^{2+} (e.g., coastal freshwaters), and aquatic life would not be adequately protected. The higher the Mg^{2+} levels of the water, the more eq. 3 would overestimate the total allowable Zn levels. For example, eq. 3 would calculate that in the control soft water used in the LC_{50} trials (Table 1), 40 μg $Zn{\cdot}L^{-1}$ would be the total allowable limit, and presumably protective against acute Zn toxicity to aquatic life. The 96-h LC_{50} was determined to be 103 μg $Zn{\cdot}L^{-1}$ in soft water (Fig. 6A), and in this case, eq. 3 may be protective. With the addition of 1.0 mM Ca^{2+} to soft water, eq. 3 would calculate a total allowable limit to be 343 μg $Zn{\cdot}L^{-1}$, while the LC_{50} was 1800 μg $Zn{\cdot}L^{-1}$, evidently protective. However, with the addition of 1.0 mM Mg^{2+}, eq. 3 would similarly calculate a total allowable limit to be 343 μg $Zn{\cdot}L^{-1}$. This is 5.5-fold greater than the measured LC_{50} of 63 μg $Zn{\cdot}L^{-1}$ (Fig. 6A). Clearly, this may pose harmful consequences to aquatic life.

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Crue éclair de juillet 1996 dans la région du Saguenay (Québec). 1. Impacts sur la colonne d'eau de la baie des Ha! Ha! et du fjord du Saguenay

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Résumé : Suite à une crue éclair survenue en juillet 1996 dans la région du Saguenay (Québec), la partie amont du fjord du Saguenay et la baie des Ha! Ha! ont reçu des millions de tonnes de sédiment terrestre en quelques jours. Ce premier article rapporte les observations physiques, chimiques et biologiques obtenues en septembre 1996 et août 1997 pour la colonne d'eau et cherche à établir des indicateurs capables de caractériser les effets de cette crue sur la colonne d'eau de la baie et du fjord. Les données sur l'abondance et la distribution des particules en suspension ainsi que les données chimiques sur la nature de ces particules montrent bien l'influence de la crue sur la composition particulaire des eaux de la baie et du fjord. En contrepartie, les indicateurs biologiques comme l'abondance et la distribution des espèces phytoplanctoniques et zooplanctoniques mesurées en septembre 1996 diffèrent peu entre la baie et le fjord et montrent peu de différences avec les données antérieures disponibles. Par ailleurs, l'état physiologique du zooplancton constitue un très bon indicateur de l'état de santé de l'ensemble de l'écosystème pélagique.

Abstract: A flash flood, which occurred in July 1996 in the Saguenay area (Quebec), transported millions of tons of sediment to the upstream section of the Saguenay Fjord and the Baie des Ha! Ha! in a few days. This first article reports the physical, chemical, and biological observations obtained for the water column in September 1996 and August 1997 and attempts to define indicators that can adequately characterize the effects of the flood on the water column. The chemical composition, the abundance, and the distribution of suspended particles clearly show the influence of the flood on the composition of particles in the water column of the bay and the fjord 2 months and even 1 year after the flood. However, biological indicators such as the abundance and the distribution of phytoplankton and zooplankton species determined in September 1996 are only slightly different between the Bay and the Fjord and show little difference with preflood data. The zooplankton physiological state appears to be a very good indicator of the health of the whole pelagic ecosystem.

Introduction

Les zones côtières de plusieurs parties du monde sont sujettes à de multiples perturbations naturelles, souvent catastrophiques pour les populations riveraines (Raper 1993). Le réchauffement global de l'atmosphère (Schlesinger 1991; Houghton et al. 1993) couplé à des phénomènes climatiques naturels comme El Niño semblent induire de plus en plus de bouleversements météorologiques majeurs, et il est probable que la fréquence et l'intensité des tempêtes en régions côtières augmenteront au cours des prochaines décennies (Knox 1993). Les effets de crues soudaines et d'apports massifs de sédiment sur les milieux pélagiques côtiers sont pratiquement inconnus.

Entre le 19 et le 21 juillet 1996, le territoire du Saguenay a été victime d'une crue éclair causée par la chute d'environ 200 mm de pluie en quelques heures. Cette pluie exceptionnelle a provoqué la rupture de certaines digues de retenue, le débordement de réservoirs hydroélectriques et le gonflement des rivières causant l'inondation des terres basses et d'importants dégâts matériels aux habitations et aux infrastructures de nombreuses municipalités riveraines (Walsh et Bourgeois 1996). En régime normal, la rivière Saguenay a un débit mensuel moyen de l'ordre de 1600 $m^3 \cdot s^{-1}$, et les moyennes mensuelles des crues printanières dépassent rarement 3000 $m^3 \cdot s^{-1}$ (Smith et Schafer 1987). Le 22 juillet 1996, le débit de la rivière Saguenay a atteint le niveau exceptionnel de 5500 $m^3 \cdot s^{-1}$, soit environ 3,5 fois son débit estival normal (Gilbert 1997). Les données du rapport de la Commission scientifique et technique sur la gestion des barrages (Nicolet et al. 1997) montrent que le débit normal de la petite rivière à Mars se jetant à l'extrémité de la baie des

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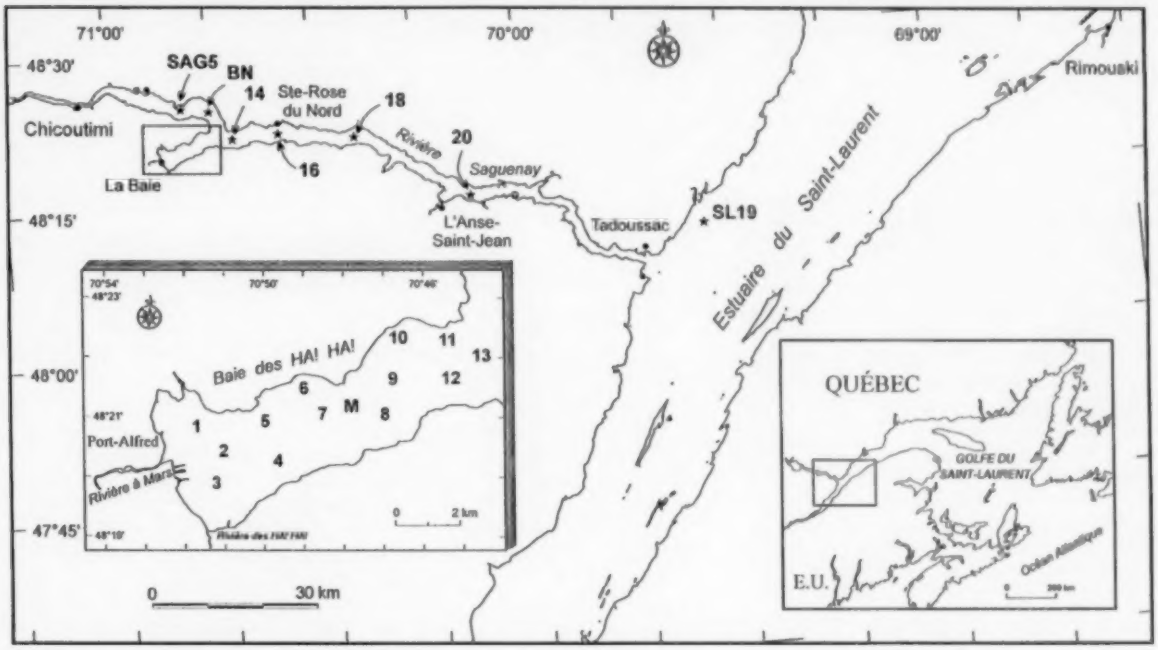
Fig. 1. Localisation des stations échantillonnées en septembre 1996 et août 1997 dans le fjord du Saguenay et l'estuaire du Saint-Laurent.



Ha! Ha! a été multiplié par 10 à 15 fois pour même dépasser 400 $m^3 \cdot s^{-1}$. La rivière Ha! Ha! se jetant aussi dans la baie des Ha! Ha! s'est transformée en un torrent de boue (>1000 $m^3 \cdot s^{-1}$ à l'embouchure) lorsque la digue de retenue du lac Ha! Ha! s'est rompue sous la pression des eaux. En conséquence, des millions de tonnes de sédiment et de débris divers, incluant certains résidus pétroliers, ont été entraînés jusque dans la baie des Ha! Ha! ainsi que dans la partie amont du bras nord du fjord. Les pires hypothèses ont alors été émises quant à une catastrophe écologique majeure et une destruction de la vie dans la baie et le fjord, en particulier, à cause du faible taux d'échange des eaux avec l'estuaire maritime du Saint-Laurent.

Les observations et les résultats analytiques de deux expéditions océanographiques ont été regroupés dans une série de deux articles qui rapportent et discutent l'ensemble des données biologiques ainsi que les résultats des analyses chimiques, géochimiques et sédimentologiques obtenus afin d'établir l'ampleur de la catastrophe écologique appréhendée. Le premier de ces deux articles porte uniquement sur la colonne d'eau et fournit des données sur la composition chimique des eaux marines, la distribution et la composition chimique du matériel particulaire en suspension et la répartition des organismes planctoniques de la baie des Ha! Ha! et du fjord. De par la nature même des travaux réalisés, cet article rend disponible une importante série de données biologiques et chimiques, inédites pour le fjord du Saguenay. Le deuxième article porte sur la caractérisation des nouveaux sédiments déposés et sur les effets de ce dépôt sur la faune benthique (Pelletier et al. 1999). En plus de formuler un diagnostic clair sur le cas spécifique des écosystèmes de la baie des Ha! Ha!, ces travaux discutent du choix des divers indicateurs environnementaux, aussi bien chimiques que biologiques, permettant de formuler un tel diagnostic à court et moyen termes dans le cas d'événements similaires pouvant se produire en milieu côtier protégé, comme une baie profonde, un fjord ou un estuaire.

Matériel et méthodes

Échantillonnage

Les expéditions PDSAG96 et PDSAG97 se sont déroulées à bord du navire océanographique *Alcide C. Horth*, du 21 au 25 septembre 1996 et du 25 au 29 août 1997 respectivement. Les échantillonnages ont été effectués à 16 stations en 1996 et à 10 stations en 1997 dans la baie des Ha! Ha!, le bras nord et le bassin profond de fjord du Saguenay (fig. 1). Pour chacune des stations, l'eau a été échantillonnée à l'aide d'une rosette munie de bouteilles Niskin de 5 L (avec thermomètres à renversement) aux profondeurs 0, 2, 5, 10, 50, 100, 150 m et à 20 m du fond pour les stations les plus profondes. L'eau des bouteilles Niskin a été sous-échantillonnée pour les mesures ultérieures de la salinité, des sels nutritifs, du matériel particulaire en suspension, des pigments algaux, ainsi que pour l'identification et la détermination de l'abondance des espèces du phytoplancton et du microzooplancton. Les échantillons de mésozooplancton ont été récoltés à l'aide de traits verticaux (de 15 m du fond jusqu'à la surface) d'un filet ayant un vide de maille de 202 mm aux stations 2, 7, 9 et 13 en 1996 seulement. Ces échantillons biologiques ont été conservés dans une solution de formol boraté – eau de mer à 4% à l'exception du microzooplancton (Lugol acide 0,4%).

Analyses en laboratoire

Des sous-échantillons d'eau (250 à 500 mL) ont été prélevés sans tamisage et préservés par l'ajout de Lugol acide (concentration finale 3%) pour le comptage ultérieur des particules en laboratoire à l'aide d'un compteur Coulter, modèle TA2, muni alternativement d'un tube de 70 ou 280 µm comme diamètre d'ou-

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verture de l'orifice. L'abondance et la masse volumique des particules ($\mu L \cdot L^{-1}$) ont été déterminées pour 23 classes de taille réparties entre 1 et 180 µm. Il est à noter que la préservation des échantillons au Lugol peut affecter la distribution des particules en nombre et en volume (Olivieri 1985). La contraction des cellules provoquent un accroissement du nombre et de la masse volumique des cellules de petite taille. Étant donné que les échantillons ont été traités quelques jours après l'expédition, les changements qui ont pu se produire devraient représenter un faible pourcentage de l'ensemble des particules.

Les échantillons pour les analyses des sels nutritifs ont été filtrés (<13 kPa) à travers des filtres Whatman GF/F (25 mm) préalablement rincés avec 50 mL d'eau de mer. Les filtrats ont été conservés à –20°C dans des cryoviales en polypropylène pré-lavées au HCl 10% (v/v) pour la détermination ultérieure des concentrations en nitrites + nitrates, phosphates et silicates à l'aide d'un autoanalyseur FSIII de Perstorp (Alpkem, O.I. Analytical). Des échantillons d'eau (500 mL) ont été filtrés sur des filtres Whatman GF/F (brûlés à 500°C pendant 6 h) et congelés à –20°C pour l'analyse ultérieure du carbone et de l'azote organique particulaire au moyen d'un analyseur CHN de Perkin-Elmer ainsi que pour la détermination de la masse totale des particules en suspension ($\mu g \cdot L^{-1}$).

Les concentrations de chlorophylle (Chl) *a* et des phéopigments ont été déterminées par fluorimétrie avant et après acidification (HCl 5% v/v) selon la méthode de Parsons et al. (1984). Les échantillons (250 mL) pour ces analyses ont été filtrés sur des filtres Whatman GF/F (porosité : 0,7 µm) ainsi que sur des filtres en polycarbonate Nuclepore (porosité : 5 µm). Les filtres ont été ensuite extraits dans des fioles à scintillation préalablement remplies avec 10 mL d'acétone 90% et placés à 0°C dans l'obscurité pendant au moins 18 h.

Les concentrations des différents pigments algaux, recueillis sur des filtres Whatman GF/F, pour les profondeurs de 0 et 2 m pour les stations 2, 7, 9 et 13 (en 1996) ont été déterminées par chromatographie liquide (HPLC) selon une méthode adaptée de Wright et al. (1991). Les filtres ont été d'abord extraits avec un mélange méthanol – acétate d'ammonium (98:2), les pigments ont été ensuite séparés sur une colonne Spherisorb ODS-2 (5 µm) (150 × 4,6 mm d.i.) et quantifiés à l'aide d'un spectrophotomètre à barette de diodes Waters 991 et d'un spectro-fluorimètre ABI Spectroflow 980. Les étalons externes de la Chl *a* et *b* ainsi que la β-carotène ont été obtenus de la compagnie Fluka, tandis que les autres étalons ont été séparés et purifiés en laboratoire par chromatographie sur couche mince (Roy et al. 1996).

La détermination de l'abondance et de la taille des cellules phytoplanctoniques a été réalisée par l'analyse flux cytométrique de 24 échantillons prélevés en 1996 aux stations 2, 7, 9, 13 et 18, fixés au paraformaldéhyde (2%) et congelés rapidement dans l'azote liquide. Le cytomètre FASCSort de Becton Dickinson a été réglé aux longueurs d'onde de fluorescence FL1 (530 nm), FL2 (585 nm) et FL3 (650 nm). Les canaux FL2 et FL3 ont été attribués respectivement à la fluorescence de la phycoérythrine et de la Chl *a*. Chaque échantillon a été analysé en duplicata en utilisant un volume de 275 à 350 µL. L'analyse des diagrammes de dispersion a permis de distinguer les cellules de petite taille comprise entre ~0,3 et 5 µm et les cellules de taille comprise entre 5 et ~20 µm. L'étude des cellules planctoniques a été complétée par l'analyse taxonomique d'échantillons d'eau récoltés à 5 m aux stations 2, 7, 9, 13 et 18 en 1996 selon la méthode de Utermöhl (1958). Les cellules (phytoplancton et micro-zooplancton) ont été identifiées et comptées au microscope inversé sous un grossissement de 1000×.

Pour l'étude spécifique des protozoaires ciliés, les échantillons de l'eau de surface provenant de stations 2, 7, 9, 13 et 18 en 1996 ont été préservés avec du Lugol acide (concentration finale de 0,4%). Cette concentration du préservatif suit la norme acceptée pour ces études sans un effet majeur sur le micro-zooplancton (Sherr et Sherr 1993). Trois mois après l'expédition, les échantillons ont été mis à sédimenter selon la méthode d'Utermöhl (1958). Les ciliés pélagiques ont été classifiés en deux catégories : (1) ciliés nus (sans lorica) et (2) ciliés-tintinnides (avec lorica).

Pour l'identification du méso-zooplancton, un fractionnement préliminaire sur soie de 750 µm a été effectué afin de faciliter le comptage. Les sous-échantillons de taille supérieure à 750 µm ont été entièrement comptés aux stations 2, 7, 9 et 13. Pour la fraction de taille inférieure à 750 µm, un aliquote d'environ 1% a été dénombré pour chaque station. La composition spécifique a été déterminée jusqu'aux stades copépodites pour les espèces principales et de taille supérieure à 750 µm.

Résultats et discussion

Une crue éclair du type de celle qui a frappé la région du Saguenay en juillet 1996 est tout à fait exceptionnelle dans les annales météorologiques canadiennes, et il n'existe à notre connaissance aucun cas documenté sur les effets d'une telle crue sur un milieu côtier. Le cas du fjord du Saguenay est unique parce qu'il offre la possibilité d'évaluer les impacts environnementaux d'un événement catastrophique terrestre sur un milieu côtier ayant des échanges limités avec le large et dont les frontières géographiques et océanographiques sont bien délimitées. Les résultats de 1996 et 1997 ont été regroupés et ordonnés de façon à décrire d'abord les données physiques, ensuite les données chimiques concernant l'eau et les particules et enfin les données biologiques sur le plancton. Les divers paramètres mesurés sont présentés comme des indicateurs potentiels d'un événement catastrophique récent, et des comparaisons avec des données antérieures ont été établies à la mesure des résultats disponibles.

1. Les eaux du fjord et de la baie

Les tout premiers paramètres susceptibles d'avoir été influencés par la crue éclair sont ceux de la température et de la composition chimique des eaux du fjord, puisque celui-ci a reçu en plein milieu de l'été un volume d'eau douce équivalent à celui d'une crue printanière. Il importait donc de déterminer si l'effet de dilution était encore perceptible quelques semaines après les événements.

Caractéristiques physico-chimiques des eaux

Le caractère fortement stratifié de la masse d'eau du fjord du Saguenay a été décrit par plusieurs auteurs (Drainville 1968; Schafer et al. 1990) qui ont montré la présence d'une pycnocline permanente séparant une couche d'eau saumâtre d'au plus 20 m provenant de la dilution progressive de l'eau douce des affluents avec l'eau salée sous-jacente, d'une masse d'eau plus dense (beaucoup plus salée et plus froide) provenant de la couche intermédiaire de l'estuaire du Saint-Laurent. Les données spécifiques à la baie des Ha! Ha! sont rares et le régime de circulation y est mal connu. Afin de tenter de déterminer l'impact de l'inondation sur la stratification, nous avons comparé quelques profils CTD recueillis avant (juin 1996) et après (septembre 1996) les événements. Cette année-là, la couche de surface (0–5 m) est mieux définie et la couche intermédiaire (5–50 m) est plus salée en septembre qu'en juin (fig. 2). Entre 10 et 40 m, les différences de température varient entre 2 et 0,2°C. Cependant, cette différence pourrait être attribuée à la variation inter-annuelle. En effet, des données obtenues de juin 1992 (non présentées) montrent une couche de surface de plus de 10 m

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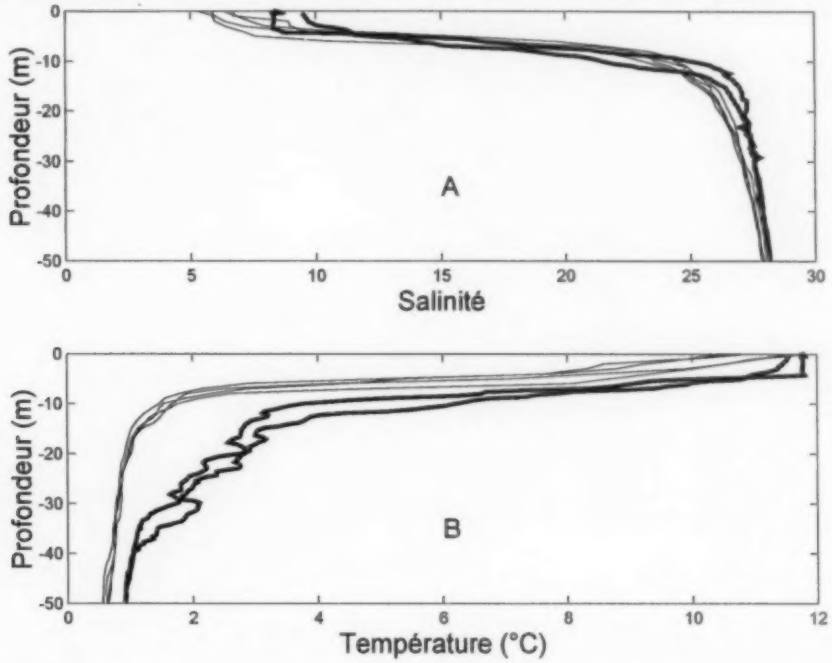
Fig. 2. Salinité et température dans les premiers 50 m de la colonne d'eau au centre de la baie des Ha! Ha!. Les deux lignes épaisses correspondent au stations 8 (marée basse) et 9 (marée haute) de septembre 1996, tandis que les lignes minces correspondent à quatre autres profils obtenus à la station 7 à 12 h d'intervalle en juin 1996 (Y. Gratton, données non publiées), 1 mois avant la crue spontanée.



d'épaisseur, tandis que sous les 15 m, les profils de juin 1992 sont similaires à ceux de juin 1996, aux mêmes stations.

Des données physiques et chimiques plus détaillées des eaux du fjord sont présentées au tableau 1. Afin de mettre en évidence le contraste existant entre la couche de surface et la masse d'eau profonde, des valeurs moyennes ont été calculées pour la couche 0–5 m et pour la couche plus profonde que 50 m. De plus, des valeurs moyennes pour l'ensemble des stations de la baie des Ha! Ha! et pour celles du bassin profond du fjord ont été calculées pour permettre la comparaison des deux secteurs échantillonnés.

Les températures moyennes de la couche de surface dans la baie et dans le fjord étaient identiques (11,6°C) en septembre 1996, tandis que la couche de fond semblait plus froide dans la baie (1,2°C) que dans le fjord (1,7°C). Cette apparente anomalie pourrait simplement être reliée au calcul des moyennes qui s'est effectué sur des profondeurs plus faibles dans la baie (50–150 m) et plus grandes dans le bassin profond (50–250 m). Les eaux profondes des bassins du fjord étant légèrement plus chaudes que celles de la couche intermédiaire qui entrent dans la baie, les moyennes et les écarts-types en sont légèrement augmentés. Les salinités moyennes en surface sont similaires dans la baie (9,6 ± 2,5) et le fjord (10,1 ± 1,4) et ne montrent aucun effet de dilution dans la baie. Les valeurs observées sont comparables à celles rapportées pour l'automne dans le fjord (Schafer et al. 1990). Les données de salinité et température pour août 1997 sont présentées au tableau 2. Les températures des eaux de surface en 1997 sont identiques dans la baie (13,7°C) et le fjord (13,8°C) et sont environ 2°C plus élevées que celles observées en 1996, cette différence étant attribuable à la différence de saison entre les deux expéditions. Les salinités en surface et dans la couche profonde sont légèrement plus élevées en 1997 qu'en 1996 sans que ces différences soient significatives.

L'ensemble des données de salinité et de température (tableau 1 et fig. 2) montre que même si les apports massifs d'eau douce de juillet 1996 ont pu modifier à court terme la stratification du fjord, ces effets n'étaient plus discernables à la fin septembre 1996.

Sels nutritifs

De façon générale, les eaux intermédiaires de l'estuaire du Saint-Laurent à l'embouchure du Saguenay sont riches en phosphates (1,2–1,4 µM) et nitrates (10–14 µM) mais pauvres en biomasse algale (Chl *a* <1 µg·L^{-1}) (Therriault et Lacroix 1976). Selon les données publiées par Côté et Lacroix (1978*a*, 1979), les concentrations en phosphates et nitrates des eaux de surface fluctuent peu dans la partie amont du fjord et demeurent nettement plus faibles que dans la partie aval où les variations saisonnières sont plus nettes. Ainsi, pour des échantillons prélevés dans le bras nord du fjord (à proximité de l'embouchure de la baie des Ha! Ha!) en octobre 1972, les concentrations en phosphates dans la couche de surface étaient légèrement inférieures à 0,5 µM, alors que les concentrations dans la couche profonde étaient de l'ordre de 1 µM. Les concentrations en nitrates pour la même station à la même période étaient de 6 à 7 µM en surface et entre 10 et 15 µM en profondeur (Côté et Lacroix 1978*a*).

Les valeurs en phosphates et nitrates obtenues pour la baie et le fjord en septembre 1996 (tableau 1) sont assez proches des données antérieures. Nos valeurs moyennes dans les

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Tableau 1. Sommaire des variables physiques et chimiques permettant de caractériser les eaux de la baie des Ha! Ha! et du fjord du Saguenay en septembre 1996.

Station	Prof. (m)	Temp. (°C)	Salinité	Masse des particules ($mg \cdot L^{-1}$)	Volume des particules ($\mu L \cdot L^{-1}$)	Chl *a* totale ≥0,7 µm ($\mu g \cdot L^{-1}$)	Phéopig. ≥0,7 µm ($\mu g \cdot L^{-1}$)	Nitrates + nitrites (µM)	Phosphates (µM)	COP ($\mu g \cdot L^{-1}$)	C/N (atomes)
1	0–5	11,1	9,58	6,72	2,03	0,50	0,38	7,64	0,42	133	11,8
	>50	nd	18,64	4,93	0,42	0,01	0,06	8,39	0,87	77	15,1
2	0–5	11,6	12,72	9,18	1,51	0,50	0,35	8,07	0,50	183	9,7
	>50	1,1	29,04	4,03	0,40	0,02	0,07	9,74	0,96	114	19,5
3	0–5	11,1	10,02	9,21	1,91	1,64	0,40	6,96	0,75	128	10,8
	>50	1,2	28,55	4,84	0,31	0,03	0,12	12,19	1,14	71	14,5
4	0–5	11,9	10,02	7,35	1,66	1,07	0,21	6,74	0,65	160	14,7
	>50	1,2	28,61	4,35	0,24	0,03	0,04	11,69	0,88	176	29,9
5	0–5	11,5	9,34	7,69	1,74	2,26	0,48	7,22	0,45	108	11,2
	>50	1,4	28,61	4,25	0,31	0,02	0,05	8,29	0,89	50	11,6
6	0–5	nd	nd	12,25	1,48	0,43	0,42	7,36	0,72	81	13,8
	>50	nd	nd	4,16	0,26	0,03	0,08	11,55	1,60	56	12,4
7	0–5	11,4	10,34	8,39	2,16	0,47	0,35	5,52	0,62	160	11,4
	>50	1,2	28,71	8,43	0,62	0,04	0,06	12,76	1,14	62	12,5
8	0–5	11,7	8,54	13,60	2,37	0,38	0,42	7,11	0,64	169	17,4
	>50	nd	29,21	4,37	0,37	0,06	0,07	10,35	1,21	81	17,5
9	0–5	11,6	8,64	10,46	1,96	nd	nd	7,96	0,80	155	16,1
	>50	nd	29,35	3,91	0,45	nd	nd	11,28	1,03	114	23,7
10	0–5	nd	8,92	6,12	1,84	0,64	0,45	8,39	0,58	98	15,3
	>50	nd	29,42	4,12	0,38	0,06	0,09	11,94	1,22	71	19,5
12	0–5	12,4	8,20	nd	2,11	0,37	0,69	4,77	0,37	128	13,6
	>50	1,4	28,69	nd	0,24	0,05	0,07	9,14	1,11	101	17,7
13	0–5	11,9	9,09	5,70	1,85	0,61	0,43	6,31	0,59	123	14,5
	>50	nd	29,44	6,89	0,20	0,05	0,06	9,01	1,44	99	23,7
Moyenne ± écart-	0–5	11,6 ± 0,4	9,6 ± 1,2	8,8 ± 2,5	1,80 ± 0,25	0,81 ± 0,61	0,42 ± 0,12	7,0 ± 1,1	0,59 ± 0,13	136 ± 31	13,4 ± 2,4
type St. 1 à 13	>50	1,2 ± 0,1	28,93 ± 0,36	4,9 ± 1,4	0,35 ± 0,12	0,04 ± 0,02	0,07 ± 0,02	10,5 ± 1,6	1,12 ± 0,22	89 ± 35	18,1 ± 5,5
14	0–5	11,8	9,19	6,44	1,70	0,45	0,39	6,16	0,53	103	11,9
	>50	1,5	28,89	5,67	0,26	0,10	0,11	9,63	1,28	66	14,8
16	0–5	11,8	8,77	8,18	1,97	0,77	0,40	5,16	0,37	103	11,1
	>50	1,4	29,04	5,49	0,42	0,11	0,09	10,56	1,18	54	12,1
18	0–5	11,4	10,60	10,19	1,65	0,62	0,39	6,02	0,43	125	15,1
	>50	1,7	29,25	6,09	0,49	0,70	0,28	12,07	0,80	94	16,2
20	0–5	11,4	11,82	5,96	1,19	0,37	0,29	7,07	0,50	94	11,1
	>50	2,4	29,32	5,15	0,51	0,56	0,23	8,34	1,09	73	10,8
Moyenne ± écart-	0–5	11,6 ± 0,2	10,1 ± 1,4	7,7 ± 1,9	1,63 ± 0,32	0,55 ± 0,18	0,37 ± 0,05	6,10 ± 0,78	0,46 ± 0,01	106 ± 13	12,3 ± 1,9
type St. 14 à 20	>50	1,7 ± 0,4	29,12 ± 0,20	5,60 ± 0,3	0,42 ± 0,11	0,37 ± 0,31	0,18 ± 0,0	10,2 ± 1,6	1,09 ± 0,21	72 ± 17	13,5 ± 2,5

Nota : Les valeurs sont des concentrations moyennes calculées à partir de trois mesures dans la zone 0–5 m et de deux mesures dans la zone de plus de 50 m. nd, valeur non déterminée; COP, carbone organique particulaire.

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eaux de surface ne diffèrent pas entre la baie et le fjord, quoique les valeurs dans la baie soient en général légèrement plus élevées. Cette tendance à un léger enrichissement de l'aval vers l'amont n'est pas présente dans les données de Côté et Lacroix (1978*a*) et va à l'encontre de l'idée généralement admise que les eaux du fjord s'appauvrissent progressivement au cours de leur remontée lente vers l'amont du fjord. Cet enrichissement vers l'amont semble propre à la situation de septembre 1996 et pourrait s'expliquer par une contribution exceptionnelle et temporaire provenant de la décomposition de matière organique terrestre apportée par la crue.

Le rapport entre les concentrations en nitrates et phosphates de la baie des Ha! Ha! montre un coefficient de détermination assez faible (r^2 = 0,306) et la valeur moyenne N/P est d'à peine 6,4:1. Ce rapport est bien inférieur aux valeurs observées dans l'estuaire du Saint-Laurent à l'embouchure du Saguenay (environ 10:1 selon les données de Therriault et Lacroix 1976). Notre rapport moyen N/P est aussi inférieur aux valeurs rapportées par Côté et Lacroix (1978*a*) qui trouvent pour le Saguenay environ 8,5:1 dans les eaux de surface et environ 12:1 dans les eaux profondes avec d'importantes fluctuations associées aux mouvements de la marée.

Un rapport N/P faible dans certains estuaires ou fjords est le plus souvent interprété comme un apport terrigène plus élevé en phosphore inorganique qu'en nitrates dû en particulier à l'oxydation des polyphosphates utilisés comme détergents et rejetés par les eaux usées urbaines. Le lessivage des sols agricoles et le débordement des égouts municipaux pendant la crue pourraient être responsables d'un apport important de phosphates susceptibles de déséquilibrer pour au moins quelques semaines la répartition des sels nutritifs dans la baie des Ha! Ha!.

Enfin, notons une grande fluctuation des concentrations en silicates mesurées dans la couche de surface de la baie (39,9 ± 8,9 µM) et du fjord (36,1 ± 11,7 µM) (résultats non montrés), qui semble indiquer un enlèvement partiel des silicates dissous dans les eaux de surface de la baie. Il est possible que l'apport massif de particules d'argile avec la crue éclair ait pu accentuer le mécanisme d'enlèvement des silicates, mais les données actuelles et antérieures sont insuffisantes pour conclure. L'analyse des sels nutritifs nous a permis de dégager les premiers indicateurs d'une perturbation chimique des eaux de la baie des Ha! Ha! qui étaient anormalement enrichies en phosphates et montraient un rapport N/P faible 2 mois après la crue. Cependant, il faut noter le caractère transitoire de ces indicateurs et la nécessité de les appliquer à court terme. Deux mois représentaient probablement la limite de leur utilisation efficace. Pour cette raison, nous n'avons pas répété ces mesures en 1997 ayant peu de chance de les relier à la crue.

2. La matière particulaire en suspension

La composition et l'abondance de la matière particulaire en suspension (MPS) dans les eaux du fjord ont fait l'objet de quelques études antérieures (Sundby et Loring 1978; Chanut et Poulet 1979; Tremblay et Gobeil 1990) permettant la comparaison avec les données de 1996 et 1997. En terme de masse de particules par unité de volume, on observe en 1996 dans la couche de surface (0–5 m) de la baie une valeur moyenne de 8,8 ± 2,5 mg·L^{-1}, avec une valeur maximale de 13,6 mg·L^{-1} à la station 8 (tableau 1). En 1997, cette valeur moyenne en surface dans la baie chute à 4,7 ± 1,7 mg·L^{-1} avec une valeur maximale de 6,7 mg·L^{-1} à la station 2 (tableau 2). La différence entre les deux années est significative ($P \leq 0,01$) et indique la présence de particules terrigènes fines dans la couche de surface en septembre 1996 en concentration presque deux fois plus élevée qu'en août 1997. Il n'existe pas de données publiées pour la baie des Ha! Ha! qui soient antérieures à la crue de 1996, mais les résultats disponibles pour la couche de surface du fjord (bras nord et fjord profond) montrent des concentrations en MPS ne dépassant pas 2 à 3 mg·L^{-1} en été et automne mais pouvant dépasser 10 mg·L^{-1} au printemps (Sundby et Loring 1978; Tremblay et Gobeil 1990). Les concentrations en MPS de septembre 1996 dans la baie présentent donc une anomalie significative qui pourrait bien être une conséquence directe de la crue de juillet 1996.

Dans la couche profonde (>50 m), la masse de la MPS varie très peu pour les deux années et les valeurs moyennes se situent toujours entre 4,7 et 5,8 mg·L^{-1} indiquant de nouveau la grande homogénéité de cette masse d'eau dans la baie et le fjord pour les deux années successives. Il faut noter cependant que ces concentrations en profondeur sont de 10 à 20 fois plus élevées que les valeurs rapportées par Sundby et Loring (1978) pour des échantillonnages dans le fjord en mai et septembre 1974 ainsi que celles publiées par Tremblay et Gobeil (1990) pour des échantillons prélevés à l'été 1986.

Les profils verticaux des concentrations moyennes de la MPS pour la baie et le fjord en septembre 1996 ainsi que pour le fjord en août 1997 sont présentés à la fig. 3. Juste en-dessous des concentrations maximales de surface, on trouve généralement entre 20 et 50 m les concentrations les plus faibles, tandis qu'une augmentation significative ($P \leq 0,01$) apparaît dans la couche plus profonde. Une augmentation des concentrations en MPS dans une masse d'eau profonde est généralement interprétée comme la résultante d'une remise en suspension des sédiments du fond. Dans le cas présent, l'abondance en MPS dans les eaux profondes du fjord en septembre 1996 et même en août 1997 peut être attribuée à la crue éclair de juillet 1996. En effet, une quantité considérable de matériel particulaire fin a été déposée sur le fond de la baie et du fjord durant une courte période de temps (Pelletier et al. 1999); un tel sédiment non consolidé peut être soulevé et remis en suspension par de faibles énergies provenant de courants de fond, du déplacement sous-marin de ces sédiments (turbidités et éboulements le long des parois submergées du fjord) et de l'activité des organismes benthiques et épibenthiques.

Les données du volume total de MPS par unité de volume d'eau dans la baie viennent appuyer les données en concentrations massiques précédentes avec des valeurs moyennes en surface (1,80 ± 0,25 µL·L^{-1} en 1996 et 1,42 ± 0,48 µL·L^{-1} en 1997) bien supérieures à celles mesurées au fond (0,35 ± 0,12 µL·L^{-1} en 1996 et 0,21 ± 0,06 µL·L^{-1} en 1997) (tableaux 1 et 2). Les volumes moyens dans la couche de fond de la baie et du fjord en 1996 sont aussi nettement supérieurs à ceux mesurés par Chanut et Poulet (1979) dans la couche profonde du fjord en septembre 1974 où les volumes étaient inférieurs à 0,2 µL·L^{-1}.

L'examen comparatif des spectres de taille enregistrés en 1996 (tableau 3) montre d'abord la similarité des distribu-

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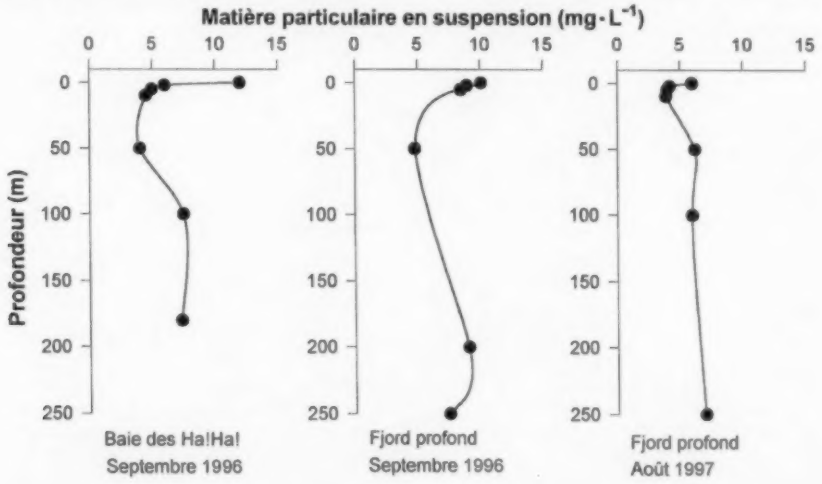
Tableau 2. Sommaire des variables physiques et chimiques obtenues sur les eaux de la baie des Ha! Ha!, du bras Nord (station BN) ainsi que du bassin profond et de l'embouchure du fjord en août 1997.

Station	Profondeur (m)	Temp. (°C)	Salinité	Masse des particules ($mg \cdot L^{-1}$)	Volume des particules ($\mu L \cdot L^{-1}$)	COP ($\mu g \cdot L^{-1}$)	C/N (atomes)
2	0–5	14,2	9,97	6,73	2,06	471	7,9
	≥50	0,9	29,28	3,96	0,29	76	10,1
7	0–5	14,1	10,03	4,88	1,12	283	8,3
	≥50	0,9	29,56	3,68	0,17	71	11,5
9	0–5	14,3	9,03	4,64	1,53	221	6,4
	≥50	1,1	29,67	5,09	0,21	11	nd
13	0–5	12,1	12,81	2,65	0,98	230	10,5
	≥50	1,3	29,52	6,03	0,15	91	12,4
Moyenne ± écart-type St. 2 à 13	0–5	13,7 ± 1,1	10,5 ± 1,6	4,73 ± 1,67	1,42 ± 0,48	300 ± 120	8,3 ± 1,6
	≥50	1,0 ± 0,2	29,51 ± 0,16	4,69 ± 1,08	0,21 ± 0,06	62 ± 35	11,3 ± 1,2
BN	0–5	7,8	7,82	4,23	1,22	276	10,5
	≥50	1,2	29,38	5,53	0,17	86	10,4
14	0–5	13,6	9,19	4,90	1,34	266	9,9
	≥50	0,9	29,12	6,05	0,24	95	11,1
16	0–5	13,9	10,60	4,42	0,91	168	8,1
	≥50	1,4	29,63	5,76	0,24	37	nd
18	0–5	13,8	11,75	4,42	1,13	228	8,3
	≥50	1,7	29,70	5,90	0,17	78	8,9
Moyenne ± écart-type St. 14 à 18	0–5	13,8 ± 0,2	10,5 ± 1,3	4,42 ± 0,00	1,13 ± 0,22	221 ± 49	8,8 ± 1,1
	≥50	1,3 ± 0,4	29,48 ± 0,32	5,83 ± 0,10	0,22 ± 0,04	70 ± 30	10,0 ± 1,5

Nota : Les valeurs sont des concentrations moyennes calculées à partir de trois mesures dans la zone 0–5 m et de deux à trois mesures dans la zone de 50 m et plus.

Fig. 3. Profils verticaux de la matière particulaire en suspension (MPS) : baie des Ha! Ha! en septembre 1996 (valeurs moyennes des stations 2, 7, 9 et 13); fjord profond en septembre 1996 et août 1997 (valeurs moyennes des station 14, 16 et 18).



tions de taille dans les eaux de surface de la baie des Ha! Ha! (stations 2, 9 et 13), distributions dominées par les petites particules de taille entre 2,9 et 18 µm. Quant aux eaux de surface des stations 16 et 18 du fjord, elles sont dominées par des particules encore plus fines appartenant aux tailles de 2,9 à 5,8 µm. La situation en août 1997 dans les eaux de surface est très différente (tableau 3) avec une distribution beaucoup plus hétérogène et la prédominance dans tous les spectres de particules très fines correspondant à la taille des argiles (1,0–1,8 µm) et de particules de tailles intermédiaires (9–18 µm), pouvant correspondre à certains types de cellules planctoniques (p. ex., flagellés, diatomées). Dans la couche profonde, on observe peu de variations inter-annuelles avec des distributions très similaires de 1996 à 1997 pour chacune des stations échantillonnées. Les différences spatiales ne sont pas plus évidentes, mais on remarque tout de même une proportion plus importante de particules fines (≤18 µm) en 1996 dans les stations de la baie probablement due à la resuspension. On trouve d'autre part de grosses particules dans les stations du fjord, phénomène pouvant être relié à la flo-

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Tableau 3. Distribution des particules en suspension (% du total) par classes de tailles dans la couche de surface (0–5 m) et dans la couche profonde (>50 m) de la baie des Ha! Ha! et du fjord du Saguenay en septembre 1996 et en août 1997.

Classes de tailles (µm)	Station					
	2	9	13	14	16	18
Couche de surface (0–5 m) 1996						
1,0–1,8	9	10	12	13	13	13
1,9–2,8	8	5	5	9	12	12
2,9–5,8	20	18	21	22	32	28
5,9–9,0	17	23	23	21	17	17
9,1–18,0	26	29	30	23	13	11
18,1–28	9	7	5	4	4	3
28,1–58,0	8	5	3	5	5	5
58,1–90	3	3	1	3	3	3
90,1–180	<1	<1	<1	<1	1	8
Couche de surface (0–5 m) 1997						
1,0–1,8	14	23	18	25	25	19
1,9–2,8	6	10	6	11	10	8
2,9–5,8	10	16	13	20	16	13
5,9–9,0	13	13	13	15	13	12
9,1–18,0	21	21	21	17	19	21
18,1–28	10	6	11	6	8	13
28,1–58,0	14	5	10	3	6	6
58,1–90	4	2	3	2	2	2
90,1–180	8	4	5	1	1	6
Couche de fond (≥ 50 m) 1996						
1,0–1,8	5	4	8	4	3	4
1,9–2,8	7	5	9	3	4	5
2,9–5,8	16	14	19	9	9	11
5,9–9,0	14	15	16	11	9	10
9,1–18,0	22	24	23	28	15	18
18,1–28	12	11	11	17	9	11
28,1–58,0	14	12	9	18	18	15
58,1–90	10	12	4	10	11	10
90,1–180	<1	3	1	<1	22	16
Couche de fond (≥ 50 m) 1997						
1,0–1,8	4	4	6	5	5	5
1,9–2,8	4	5	6	5	5	6
2,9–5,8	9	16	18	12	10	14
5,9–9,0	8	13	16	12	11	12
9,1–18,0	17	18	26	24	16	17
18,1–28	14	13	14	12	11	13
28,1–58	18	11	12	17	12	15
58,1–90	16	7	2	7	5	7
90,1–180	10	13	<1	6	25	11

culation d'une partie de la MPS fine transportée en surface. Il faut noter que la distribution des particules dans la couche intermédiaire de la station SL19 (non présentée) est dominée par les tailles 9 à 58 µm et qu'une partie de ces eaux pénètre régulièrement dans le bassin profond du Saguenay et vient donc y augmenter la concentration des particules.

La matière particulaire en suspension constitue de toute évidence un indicateur clair des perturbations entraînées par la crue, perturbations toujours perceptibles 1 an plus tard à cause de la resuspension des particules les plus fines. Les spectres de taille offrent une trop grande variabilité spatiale et temporelle et sont de ce fait plus difficiles à interpréter en fonction d'un événement catastrophique. Ce paramètre serait probablement beaucoup plus utile à quelques jours des événements alors qu'une bonne partie des particules les plus fines sont encore en suspension dans la masse d'eau à étudier.

Carbone organique particulaire et le rapport C/N

La mesure du carbone organique particulaire (COP) fournit certaines indications sur la nature de la MPS recueillie dans la colonne d'eau du fjord en septembre 1996 et en août 1997 (tableaux 1 et 2). Les particules des eaux de surface de la baie et du fjord étaient nettement plus pauvres en COP en 1996 (128 ± 30 $\mu g \cdot L^{-1}$ pour toutes les stations) qu'en 1997 (268 ± 90 $\mu g \cdot L^{-1}$), alors que la masse des particules par unité de volume y était pratiquement le double. Ainsi, on peut calculer que la concentration en COP par unité de masse de MPS fluctuait entre 13 et 18 mg $C \cdot g^{-1}$ tant en surface qu'en profondeur dans la baie comme dans le fjord en 1996, alors que les eaux de surface de la baie (64 mg $C \cdot g^{-1}$) et du fjord (50 mg $C \cdot g^{-1}$) transportaient des particules bien plus riches en carbone organique en 1997. L'ensemble des eaux profondes de la baie et du fjord montrent une valeur moyenne de COP (68 ± 29 $\mu g \cdot L^{-1}$) comparable à celle de 1996 (85 ± 32 $\mu g \cdot L^{-1}$). Les données de COP fournies par Therriault et al. (1984) pour une station échantillonnée en août 1978 en aval de notre station 20 indiquent des valeurs de l'ordre de 150 à 200 $\mu g \cdot L^{-1}$, ce qui semble nettement plus élevé que nos valeurs moyennes. Ces résultats soulignent le caractère plutôt minéral (présence d'argile et de silt) du matériel particulaire se trouvant dans les eaux de surface de la baie en 1996 par rapport au caractère plus organique du même matériel en 1997 et confirment la préservation de ce caractère dans les eaux profondes pour les deux années échantillonnées par rapport aux données antérieures. Les rapports atomiques C/N pour septembre 1996 (tableau 1) sont généralement plus élevés pour les particules de la couche profonde de la baie (gamme de 11,6 à 29,9 avec une moyenne de 18,1 ± 5,5) par rapport aux valeurs de surface (gamme de 9,7 à 17,4 avec une moyenne de 13,3 ± 2,4). Quoique la différence entre ces deux moyennes ne soit pas significative, cette tendance à des valeurs plus élevées au fond nous indique la présence de matière organique particulaire à prédominance plutôt terrigène et supporte l'idée d'une resuspension du matériel récemment déposé. Les moyennes des rapports mesurés dans l'estuaire du Saint-Laurent à l'embouchure du Saguenay (SL19) en septembre 1996 étaient de 7,3 en surface et de 9,0 en profondeur. Les résultats de 1997 confirment une réduction générale du carbone organique terrigène par rapport au carbone marin dans tout le fjord mais confirment aussi l'idée de la resuspension encore importante dans la baie 1 an après les inondations.

Le carbone et l'azote organique particulaire sont de toute évidence de bons indicateurs des bouleversements survenus dans la baie des Ha! Ha! même 2 mois après la crue. L'ensemble des données sur l'abondance et la distribution des particules en suspension ainsi que les données chimiques sur la nature de ces particules constituent dans le cas présent de meilleurs indicateurs que les paramètres chimiques du matériel dissous (salinité et sels nutritifs).

3. Les organismes planctoniques

Étant donné les scénarios plus ou moins catastrophiques

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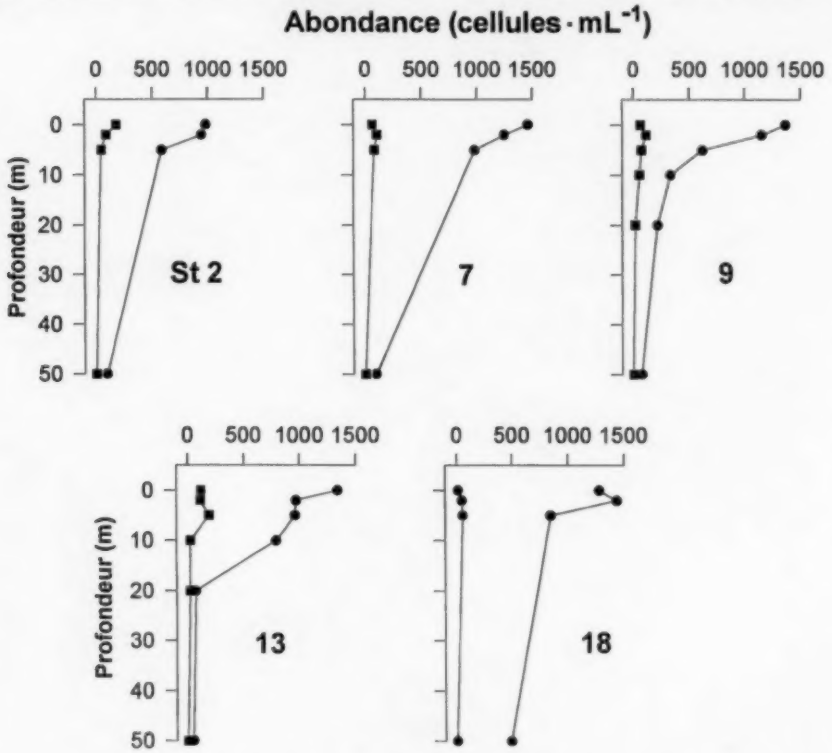
Fig. 4. Répartition verticale des cellules phytoplanctoniques dénombrées par cytométrie de flux aux cinq stations étudiées. Deux classes de taille ont été distinguées : les cellules <5 µm (●) et celles de taille comprise entre 5 et ~20 µm) (■).



avancés par certains observateurs à la suite de la crue (Walsh et Bourgeois 1996), il importait de déterminer jusqu'à quel point l'écosystème pélagique avait pu être touché par les apports massifs d'eau douce et de particules en suspension. Les efforts de caractérisation ont donc porté sur les principales espèces planctoniques de la colonne d'eau qui auraient pu être éliminées ou fortement perturbées par une brusque dilution des eaux marines ou une réduction de la zone photique par les particules terrigènes. De nouveau, les efforts ont été orientés vers la recherche d'indicateurs et non vers une description détaillée du milieu.

Abondance des cellules et pigments algaux

Une première approche a été effectuée à l'aide de la cytométrie de flux afin de déterminer la distribution de taille et l'abondance des cellules phytoplanctoniques. La figure 4 présente la répartition verticale des cellules phytoplanctoniques aux cinq stations étudiées. Les cellules <5 µm représentaient environ 90% du nombre total de cellules phytoplanctoniques de taille inférieure à 20 µm. Ces petites cellules atteignaient $1{,}4 \times 10^6$ cellules·L^{-1}, alors que les cellules entre 5 et 20 µm présentaient des abondances maximales de $0{,}18 \times 10^6$ cellules·L^{-1}. La station 2, la plus près de la Rivière des Ha! Ha! (fig. 1), présentait des abondances phytoplanctoniques légèrement plus faibles, alors que les autres stations avaient sensiblement les mêmes abondances. Il y avait généralement peu de cellules algales dans la couche aphotique, sauf pour la station 18 qui montre des abondances relativement élevées à 50 m. Ces cellules provenaient vraisemblablement des eaux de l'estuaire maritime qui pénètrent régulièrement dans le fjord. Ainsi, cette mesure par cytométrie nous fournit l'abondance du matériel phytoplanctonique inférieur à 20 µm dans la baie mais ne nous informe pas sur le fonctionnement normal de l'écosystème pélagique.

La deuxième approche a consisté à analyser la composition pigmentaire du phytoplancton par la méthode fluorimétrique traditionnelle. Le tableau 1 donne une vue d'ensemble des concentrations totales (≥0,7 µm) en Chl *a* et phéopigments retrouvées dans les eaux de surface (0–5 m) et les eaux profondes du fjord et de la baie en 1996. On observe dans la baie de fortes variations de la Chl *a* avec des valeurs maximales aux stations 3 et 5 doublant la valeur moyenne de $0{,}81 \pm 0{,}61$ µg·L^{-1}. Cette valeur moyenne est du même ordre de grandeur que celles rapportées par Côté and Lacroix (1978*a*, 1978*b*, 1979) pour la fin septembre dans le bras nord (valeurs inférieures à 0,5 µg·L^{-1}). Nos valeurs plus élevées aux stations 3 et 5 se comparent également à des données récoltées lors d'un stage de terrain du Département d'océanographie de l'Université du Québec à Rimouski en septembre 1995 au centre de la baie des Ha! Ha! (données non publiées). Les concentrations de Chl *a* analysées avec la même méthode montraient alors des maxima à 0 ou 5 m et variaient entre 1,5 et 8,0 µg·L^{-1}. Les valeurs au fond sont toujours très faibles dans la baie (≤0,06 µg·L^{-1}) alors qu'elles sont nettement plus fortes dans le fjord ($0{,}37 \pm 0{,}31$ µg·L^{-1}). Les concentrations en phéopigments montrent

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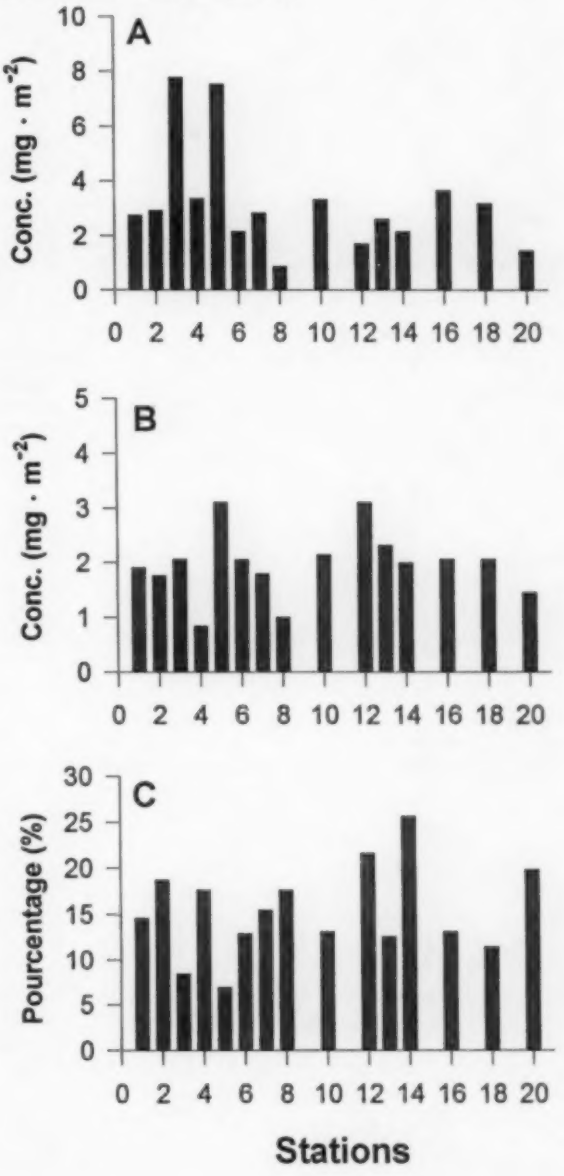
beaucoup moins de variabilité avec des concentrations moyennes en surface de 0,42 ± 0,12 $\mu g \cdot L^{-1}$ dans la baie et de 0,37 ± 0,05 $\mu g \cdot L^{-1}$ dans le fjord. On observe aussi que les phéopigments sont plus concentrés que la Chl *a* dans la couche profonde de la baie. La comparaison entre les stations des valeurs intégrées dans la couche 0–5 m montre que, mises à part les stations 3 et 5, les valeurs varient relativement peu entre les stations étudiées (fig. 5). L'influence de la marée dans ce système et le fait que les stations ont été échantillonnées à des temps et jours différents compliquent la comparaison de ces données entre elles et avec l'extérieur de la baie. De plus, ces données n'indiquent que la présence de matériel végétal et pourraient ainsi être affectées par du matériel végétal d'origine terrestre associé à la crue. Afin de préciser nos informations, nous avons eu recours, pour certains échantillons de surface, à l'analyse détaillée des pigments algaux par HPLC qui permet, entre autres, de distinguer les principaux groupes taxonomiques (Roy et al. 1996). Les résultats (tableau 4) montrent la présence de dinoflagellés (indiquée par la péridine, pigment spécifique à ce groupe), de cryptophycées (indiquée par l'alloxanthine) et probablement de diatomées (Chl $c_{1,2}$ et diadinoxanthine), mais l'absence notable de pigments appartenant aux plantes terrestres (p. ex., lutéine, Chl *b*), suggérant l'absence de matériel végétal terrestre dans les eaux échantillonnées. Les valeurs de Chl *a* rapportées ci-haut appartiennent donc bien au phytoplancton. L'autre information utile tirée de ces résultats est la caractérisation détaillée des phéopigments (Roy et al. 1996). Ceux-ci sont dominés par deux pigments généralement associés à la dégradation algale, la phéophytine *a* et la pyrophéophytine *a*, ainsi que la phéophorbide *a*, un pigment généralement retrouvé dans les produits de défécation du zooplancton herbivore. L'absence de phéopigments dérivant de la Chl *b* suggère de nouveau l'absence de végétaux terrestres. L'échantillon de 150 m à la station 13 révèle la présence de matériel algal (Chl *a*) jusqu'en profondeur (sédimentation probable).

La troisième approche de caractérisation a été l'analyse taxonomique des principales espèces phytoplanctoniques retrouvées à 5 m (tableau 5). Ici encore, ces quelques échantillons prélevés à une seule profondeur ne permettent pas une analyse fine de la taxonomie du phytoplancton dans ce milieu, le but recherché étant plutôt de détecter des indices des effets de la crue éclair. Numériquement, les groupes dominants sont les cyanobactéries suivies des chrysophycées, des chlorophycées, puis des diatomées. Les cyanobactéries sont représentées surtout par *Phormidium* sp. dans la baie et par *Chroococcus* sp. dans le fjord, deux genres fréquents en eaux douces et saumâtres (Prescott 1973). Chez les chlorophycées, un autre genre d'eau douce, *Dictyosphaerium* domine généralement à l'intérieur de la baie. Les chrysophycées sont particulièrement représentées par un genre planctonique mixotrophe, *Ochromonas*, qui semble plus abondant à l'intérieur de la baie que dans le fjord. *Falcomonas* est le genre dominant chez les cryptophycées, aussi bien dans la baie que dans le fjord. Les diatomées sont caractérisées par de nombreuses espèces appartenant aux pennales (Bacillariales), groupe fréquemment associé au domaine benthique (resuspension des sédiments ou apports des rivières). Parmi celles-ci, *Navicula* et *Nitzschia* prédominent. Une plus grande abondance de diatomées pennales vers l'intérieur de la baie

Fig. 5. Concentration (en $mg \cdot m^{-2}$) (A) de la Chl *a* intégrée entre 0 et 5 m et (B) des phéopigments intégrés entre 0 et 5 m. (C) Proportion des phéopigments par rapport aux pigments totaux exprimée en pourcentage (C) pour les stations échantillonnées en septembre 1996.



(augmentation de la station 9 vers la station 2) suggère une source riveraine peut-être associée à la crue. Enfin, chez les dinoflagellés, *Gymnodinium* est le genre dominant et son abondance est maximale à la station 18. C'est un prédateur phagotrophe qui peut ingérer de plus petites cellules appartenant au micro-zooplancton. Les autres groupes sont peu présents. Seuls les dinoflagellés, les cryptophycées et les autres groupes des chromophycées possédant de la diadinoxanthine

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Tableau 4. Pigments algaux retrouvés dans les échantillons de surface (et de 150 m pour la station 13) en septembre 1996.

Station	Profondeur (m)	Chl $c_{1,2}$ 610	Chl *a* 894	Pheid *a* 593	Phéo *a* 871	Pyrophéo *a* 813	Péridinine 631	Diadino 583	Alloxanthine 565
2	0	<ld	119,8	6,3	5,9	2,2	<ld	<ld	87,4
2	2	44,8	186,6	15,3	5,4	1,8	76,8	31,9	59,4
7	0	<ld	103,2	<ld	<ld	<ld	<ld	<ld	52,6
7	2	36,9	232,4	13,8	<ld	<ld	186,2	<ld	78,9
9	0	174,0	751,1	22,5	23,8	7,9	389,5	129,1	185,4
9	2	<ld	87,4	21,0	10,8	11,6	<ld	<ld	44,5
13	0	55,8	405,4	58,0	7,4	<ld	152,0	20,2	1935
13	2	<ld	105,8	<ld	10,3	<ld	<ld	<ld	58,0
13	150	<ld	22,4	<ld	<ld	<ld	<ld	<ld	<ld

Nota : Analyses effectuées par chromatographie HPLC. Unités, pmol·L^{-1} (multiplier par le poids moléculaire indiqué sous le nom des pigments pour transformer en pg·L^{-1}); Chl, chlorophylle; Pheid, phéophorbide; Phéo, phéophytine; Pyrophéo *a*, pyrophéophytine *a*; Diadino, diadinoxanthine; <l.d., valeur inférieure à la limite de détection. Noter que les concentrations de Chl *a* obtenues par HPLC peuvent être différentes de celles obtenues par d'autres méthodes ne discriminant pas entre les pigments.

(principalement les diatomées, les chrysophycées et les prymnésiophycées) fournissaient un signal pigmentaire détectable dans les analyses chromatographiques rapportées ci-haut. Aucune donnée taxonomique précédant l'événement de 1996 n'a pu être retrouvée concernant la baie des Ha! Ha! pour des fins de comparaison. Le seul indice taxonomique possiblement lié à la crue éclair est la présence de nombreux genres de diatomées pennales (d'origine riveraine et (ou) de la resuspension) et peut-être des cyanobactéries et chlorophycées qui semblent provenir de milieux dulcicoles. Les autres groupes répertoriés sont communément associés au phytoplancton marin.

À l'évidence, les trois approches utilisées démontrent l'abondance et la diversité de la communauté algale dans la baie des Ha! Ha! et dans le fjord 2 mois après la crue. Nous sommes donc loin d'une catastrophe qui aurait réduit la lumière au point de provoquer l'effondrement de la production primaire. Il existe trop peu de données antérieures pour juger de changements fins qui auraient pu être causés par la crue. Des données de Lee et al. (1999) obtenues en juin 1996 montrent que la communauté phytoplanctonique des eaux de surface de la baie des Ha! Ha! était principalement composée de diatomées et de flagellés (93% des espèces totales incluant les dinoflagellés), dont plusieurs genres différents de ceux répertoriés en septembre 1996. Ces différences sont plus probablement attribuables aux variations saisonnières qu'aux effets de la crue. En conclusion, les paramètres retenus dans notre étude du phytoplancton sont des indicateurs de la présence et du fonctionnement en apparence normal de la communauté phytoplanctonique du milieu étudié. Les mesures de 1996 n'ayant montré aucune perturbation majeure du phytoplancton, elles n'ont donc pas été répétées en 1997.

Micro-zooplancton

Puisque la communauté phytoplanctonique était présente et apparemment en bon état dans la baie comme dans le reste du fjord, il devenait important de pousser l'investigation et de déterminer si les autres composantes de l'écosystème pouvaient nous indiquer des effets majeurs de la crue. Les travaux ont d'abord porté sur le micro-zooplancton. Les espèces rencontrées parmi les ciliés pélagiques nus étaient : *Laboea* sp., *Strombilidium spiralis*, *Tontonia* sp., *Lohmaniella oviformis*, *Strombidium acutum*, *Strombidinopsis* sp. et *Askenasia* sp. Les tintinnides étaient des genres : *Tintinnidium*, *Stenosemella*, *Codonella*, *Ptychocylis* et *Tintinnopsis*. La figure 6 montre l'évolution spatiale de l'abondance des ciliés pélagiques nus sur la section longitudinale de la baie (station 2) et du fjord (station 18). De façon générale, leur abondance diminue de 50% de la station 2 à la station 7, puis augmente pour atteindre un maximum d'environ 8000 cellules·L^{-1} à la station 9. L'abondance des tintinnides (fig. 6) le long de la même série de stations montre une variabilité similaire à celle observée pour les ciliés nus sauf pour le maximum observé à la station 13. En général, les stations de la baie sont plus riches que celles du fjord. Certaines espèces appartenant au micro-zooplancton ont été répertoriées lors de l'énumération du phytoplancton (tableau 6). Parmi celles-ci, certains flagellés hétérotrophes tels *Bodo*, prédominant à l'intérieur comme à l'extérieur de la baie, et les choanoflagellés (*Diaphanocea* et *Salpingoeca*) numériquement plus importants en amont de la baie. Les plus fortes concentrations de l'ensemble des flagellés hétérotrophes ont été observées aux stations 7 et 9 (environ 4×10^5 cellules·L^{-1}) alors que ces concentrations étaient moins importantes aux stations 2, 13 et 18 pour s'établir autour de 3×10^5 cellules·L^{-1} (fig. 6).

Le micro-zooplancton joue un rôle indispensable dans le réseau trophique pélagique (Mostajir et al. 1999). Par leur action de prédation, les espèces micro-zooplanctoniques sont reconnues pour contrôler l'abondance des cellules phytoplanctoniques et bactérioplanctoniques. Elles sont aussi capables de régénérer l'azote et le phosphore. Le micro-zooplancton constitue donc un maillon important de la chaîne alimentaire en transférant la matière et l'énergie des niveaux trophiques inférieurs (bactéries et les petites algues) vers les niveaux trophiques supérieurs (méso- et macro-zooplancton, larves de poissons, etc.). Les données sur la répartition du micro-zooplancton dans la baie des Ha! Ha! et le fjord du Saguenay présentées ici constituent vraisemblablement les toutes premières observations du genre sans possibilité de comparaison avec d'autres études antérieures. Néanmoins, notons que quelques espèces présentes dans cette étude peuvent être rencontrées dans l'estuaire maritime du Saint-Laurent (Sime-Ngando et al. 1995). Si la population du micro-zooplancton a été directement affectée par la crue, nos résultats indiquent que ces espèces ont rapidement

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Tableau 5. Identification et énumération des cellules phytoplanctoniques recueillies à 5 m aux stations 2, 3, 7, 9, 13 et 18 en septembre 1996.

		Station					
Ordre	Genre	2	3	7	9	13	18
Chlorophycées							
Chlorococcales	*Dictyosphaerium*	120,8	—	48,8	—	322,1	—
Chlorococcales	*Monoraphidium*	—	—	6,1	—	17,9	—
Chlorococcales	*Nephroselmis*	—	—	—	201,3	—	—
Chlorococcales	*Scenedesmus*	—	—	30,5	—	—	—
Chrysophycées							
Bicosoecales	*Bicosoeca*	—	12,0	—	—	—	—
Bicosoecales	*Cafeteria*	53,7	—	12,2	—	26,8	67,1
Ochromonadales	*Ochromonas*	255,0	47,9	158,6	—	8,9	61,9
Pedinellales	*Pseudopedinella*	—	—	—	—	8,9	—
Cryptophycées							
Cryptomonadales	*Falcomonas*	67,1	95,9	61,0	26,8	98,4	31,0
Cryptomonadales	*Rhodomonas*	—	—	36,6	47,0	—	30,9
Cyanobactéries							
Chroococcales	*Chroococcus*	107,4	719,1	—	—	1932,9	20,6
Chroococcales	*Synechococcus*	—	—	—	—	402,7	—
Hormogonales	*Anabaena*	—	—	—	—	80,5	—
Hormogonales	*Phormidium*	1369,1	1498,1	—	—	—	—
Diatomées							
Bacillariales	*Achnanthes*	13,4	—	—	—	8,9	—
Bacillariales	*Asterionella*	—	—	12,2	—	—	—
Bacillariales	*Cocconeis*	13,4	—	—	—	—	—
Bacillariales	*Cymbella*	13,4	—	—	—	—	—
Bacillariales	*Fallacia*	—	—	—	—	17,9	5,2
Bacillariales	*Fragilaria*	—	12,0	—	—	—	—
Bacillariales	*Gomphonema*	13,4	—	—	—	—	—
Bacillariales	*Navicula*	40,3	—	24,4	6,7	17,9	15,5
Bacillariales	*Nitzcshia*	13,4	36,0	12,2	6,7	8,9	—
Bacillariales	*Stauroneis*	—	—	—	—	8949	—
Biddulphiales	*Melosira*	40,3	24,0	30,5	47,0	—	15,5
Biddulphiales	*Skeletonema*	—	47940	—	—	—	—
Dinoflagellés							
Gymnodiniales	*Amphidinium*	13,4	—	—	—	—	—
Gymnodiniales	*Gymnodinium*	—	12,0	—	8,1	—	588,6
Peridiniales	*Heterocapsa*	—	—	61,0	—	—	—
Prasinophycées							
Mamiellales	*Bathycoccus*	—	71,9	213,5	94,0	143,2	160,0
Prymnésiophycées							
Prymnesiales	*Chrysochromulina*	—	—	79,3	—	80,5	36,1

Nota : Unités, cellules × $10^3 \cdot L^{-1}$. Seuls les genres ont été identifiés.

recolonisé la baie des Ha! Ha! grâce à la circulation des masses d'eau dans l'axe du fjord.

Méso-zooplancton

La toute dernière étape dans la recherche d'indices sur l'état de l'écosystème de la baie et du fjord a consisté à examiner la distribution des espèces méso-zooplanctoniques. La population méso-zooplanctonique présente dans la baie des Ha! Ha! en septembre 1996 ne montre pas de variations marquées en terme de composition spécifique entre les stations (fig. 7A et 7B). La station 9 présente la plus forte abondance totale (6304 individus·m^{-3}) tout comme pour le micro-zooplancton (fig. 6). Pour la classe de taille >750 µm (fig. 7B), *Metridia longa*, une espèce omnivore, domine largement. Différentes espèces de copépodes calanoïdes représentent une part importante de l'ensemble de la population. Pour la fraction de petite taille (<750 µm), le copépode cyclopoïde *Oncaea* prédomine très nettement. Ce genre présente une concentration variant de 1000 à 3600 individus·m^{-3}. Pour l'ensemble des sites d'études, les espèces de petite taille représentent, en terme d'abondance, plus de 98% de l'ensemble de la population méso-zooplanctonique. Peu d'individus non-copépodes ont été récoltés. Les principaux taxons non-copépodes sont représentés par les chaetognathes, les appendiculaires, les ptéropodes, les amphipodes hypérides, les mysidacées et les euphausiacées (fig. 7B).

Dans son ensemble, le méso-zooplancton présent dans le fjord du Saguenay 2 mois après la crue éclair indique très

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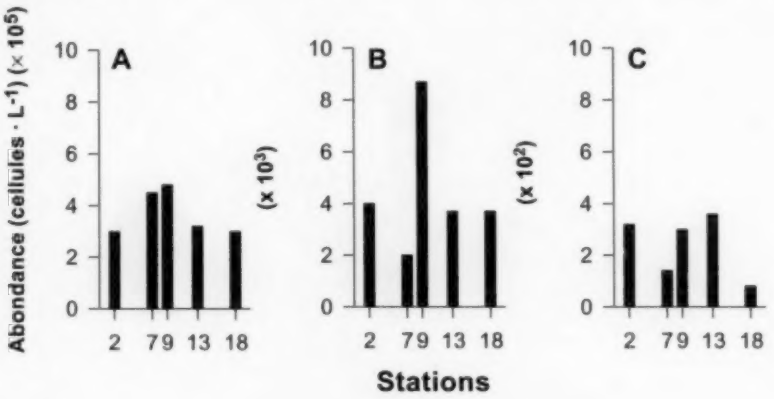
Tableau 6. Organismes du micro-zooplancton rencontrés lors des observations taxonomiques effectuées pour le phytoplancton en septembre 1996.

		Station					
Ordre	Genre	2	3	7	9	13	18
Bodonida	*Bodo*	295,3	—	384,3	463,0	277,4	278,7
Bodonida	*Cryptaulax*	—	83,9	—	—	—	—
Bodonida	*Metamonas*	—	1018,7	—	—	—	—
Kinoplastida	*Telonema*	—	36,0	30,5	—	17,9	—
Acanthoecida	*Diaphanoeca*	53,7	—	—	—	—	—
Salpingoecida	*Salpingoeca*	53,7	—	18,3	—	44,7	5,1

Nota : Unités, cellules × $10^3 \cdot L^{-1}$. Échantillons prélevés à la profondeur de 5 m.

Fig. 6. Abondance des flagellés hétérotrophes (A), des ciliés nus (B) et des tintinides (C) dans les eaux de surface de cinq stations échantillonnées en septembre 1996.



peu de variations notables par rapport aux quelques études antérieures conduites dans cette région aussi bien au niveau de la composition taxonomique que de l'abondance des différentes espèces (Rainville 1979; Gagnon et Lacroix 1982; Runge et Simard 1990). L'absence par rapport à ces travaux antérieurs de la mysidacée *Boreomysis* (organisme vivant surtout en eaux profondes) est peut-être reliée à la crue éclair, quoiqu'il soit impossible de tirer une conclusion avec le nombre restreint d'échantillons analysés. La population zooplanctonique apparaît largement dominée par les copépodes (>90%) et la contribution des petites espèces est importante. En général, la population était en bonne condition physiologique avec de nombreuses femelles de copépodes gravides (*Metridia* sp.) ou portant des sacs d'oeufs (*Euchaeta*, *Pseudocalanus*, *Oithona*, *Oncaea*). Ces observations indiquent que ces copépodes s'alimentaient normalement. De nombreux individus présentaient aussi des vacuoles de lipides, réserves développées par les espèces ayant un cycle de vie de plusieurs années.

Donc les indicateurs de l'abondance et de la diversité du micro-zooplancton et du méso-zooplancton viennent corroborer les données sur le phytoplancton en assurant que l'écosystème de la baie des Ha! Ha! et du reste du fjord n'étaient nullement sous le coup d'une catastrophe causée par la crue. Les femelles gravides des copépodes et les réserves lipidiques sont autant d'indices d'un fonctionnement normal de l'ensemble de l'écosystème à peine 2 mois après la crue.

Conclusions

L'objectif de cette étude était l'identification d'indicateurs chimiques et biologiques qui permettent d'évaluer la sévérité des dommages causés par la crue éclair à l'écosystème pélagique de la baie et du fjord. L'utilité d'un indicateur est évidemment liée à la sensibilité et à l'amplitude de sa réponse et au temps qu'il met à retrouver son équilibre. Notre étude montre que les paramètres chimiques du matériel dissous (salinité et sel nutritifs) présentent peu d'intérêt à cause d'un retour à l'équilibre trop rapide, alors que le matériel particulaire en suspension a gardé beaucoup plus longtemps l'empreinte caractéristique de la crue. Il faut en particulier noter l'effet sur la composition particulaire de la resuspension du matériel fin déposé subitement par la crue et non encore consolidé.

Au plan biologique, le choix des indicateurs est beaucoup plus difficile parce que ceux-ci sont généralement très sensibles à de multiples stresseurs environnementaux et qu'il devient rapidement difficile de discerner les effets du stresseur majeur (la crue). Il semble aussi que le temps de retour à une certaine normalité soit relativement court, vraisemblablement à cause d'une recolonisation rapide du milieu, ce qui masque les effets recherchés. Ce temps d'équilibre est évidemment lié à la circulation et à la capacité du système physique à importer de l'extérieur les éléments nécessaires à la remise en marche de l'écosystème. Dans le cas particulier

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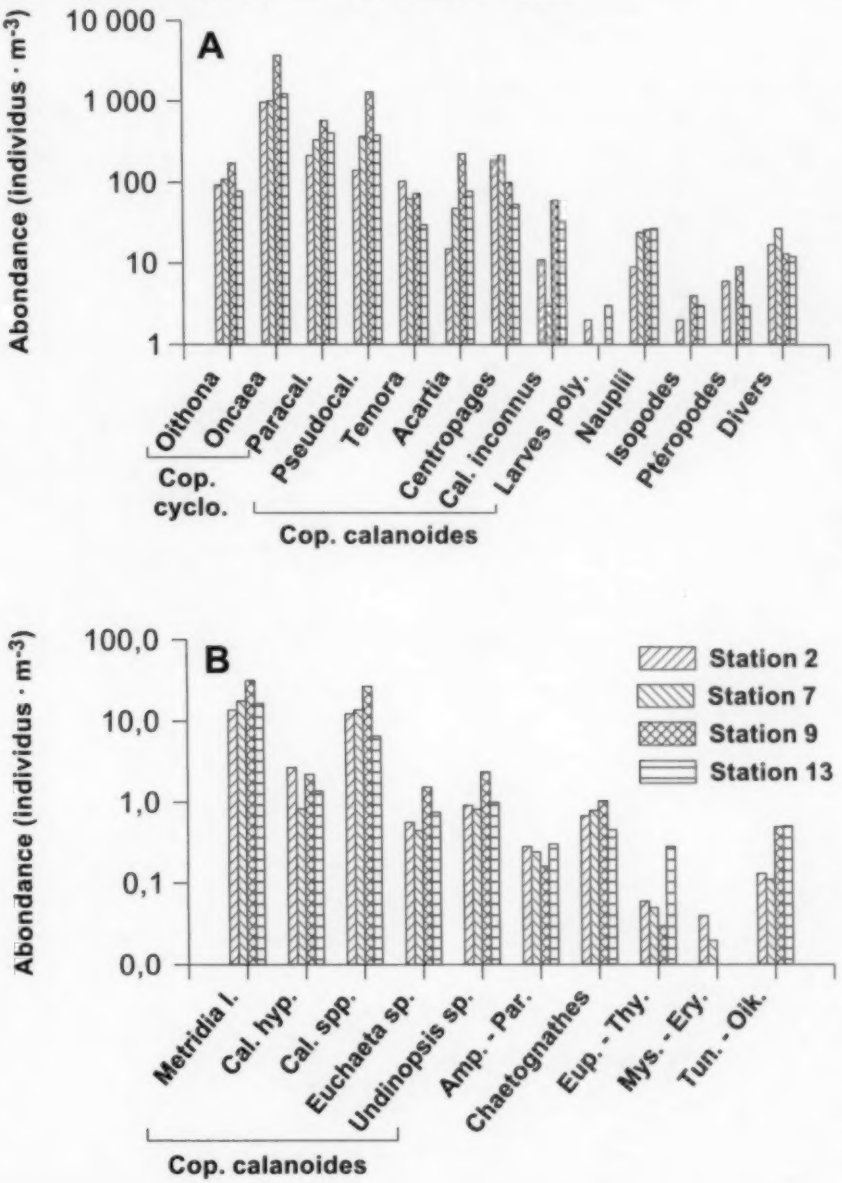
Fig. 7. Abondance (individus·m^{-3}) des principaux organismes zooplanctoniques de taille <750 µm (A) et de taille >750 µm (B) répertoriés dans la baie des Ha! Ha! et à son embouchure en septembre 1996. Abréviations : Cop. cyclo., copépodes cyclopoïdes; poly., polychètes; Cal. hyp., *Calanus hyperboreus*; Amp. – Par., amphipodes hypérides – *Parathemisto*; Eup. – Thy., euphausiacées – *Thysanoessa*; Mys. – Ery., mysidacées – *Erythrops*; Tun. – Oik., Tuniciers – *Oikopleura*.



de la baie des Ha! Ha!, cette reprise semble avoir été rapide, même si l'on considère généralement que le taux de renouvellement de ses eaux profondes est de plusieurs semaines, voir plusieurs mois.

L'ensemble de nos travaux sur la colonne d'eau de la baie des Ha! Ha! et du fjord Saguenay montre que les indicateurs les plus efficaces et aussi les plus pratiques des perturbations causées par la crue de juillet 1996 sont dans l'ordre suivant: (1) la détermination de l'abondance et de la nature chimique du matériel particulaire en suspension, (2) la teneur en chlorophylle *a* par spectrofluorimétrie et (3) la mesure de l'abondance et de l'état physiologique du méso-zooplancton. Les déterminations taxonomiques par HPLC et par microscopie fournissent des indicateurs de deuxième niveau sur l'état de

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la communauté phytoplanctonique qui sont nécessaires à des études plus fines sur la dynamique même de cette communauté.

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Crue éclair de juillet 1996 dans la région du Saguenay (Québec). 2. Impacts sur les sédiments et le biote de la baie des Ha! Ha! et du fjord du Saguenay

Émilien Pelletier, Bruno Deflandre, Christian Nozais, Guglielmo Tita, Gaston Desrosiers, Jean-Pierre Gagné et Alfonso Mucci

Résumé : Suite à une crue éclair survenue en juillet 1996 dans la région du Saguenay (Québec), plusieurs millions de tonnes de sédiments et de débris ont été transportés dans le bras nord du fjord du Saguenay et dans la baie des Ha! Ha! provoquant l'enfouissement des sédiments en place et d'une partie de l'écosystème benthique sous une nouvelle couche sédimentaire. L'objectif de ce travail est de déterminer les meilleurs indicateurs chimiques et biologiques des changements survenus au niveau de l'habitat benthique du fjord. Les nouveaux sédiments, pauvres en matière organique et riches en carbonates détritiques, contiennent de faibles teneurs de mercure et de plomb, indiquant l'absence de contamination industrielle. Le processus de stabilisation géochimique de la nouvelle couche sédimentaire a été mis en évidence par des mesures de porosité, de salinité des eaux porales et de potentiel redox (Eh) quelques semaines et quelques mois après les événements. La méiofaune a subi une réduction massive des densités moyennes des organismes par rapport aux densités enregistrées avant la crue, mais la recolonisation était déjà très nette après 1 an. D'importantes réductions d'effectifs des espèces macrobenthiques ont été enregistrées en 1997 par rapport à 1996, quoique l'abondance et la diversité du taxon des polychètes étaient généralement mieux représentées en 1997 indiquant une recolonisation de la nouvelle couche sédimentaire par des espèces pionnières.

Abstract: Several million tons of sediments and various debris were transported to the north arm of the Saguenay Fjord and the Baie des Ha! Ha! following the torrential rains and accompanying flash flood of July 1996 in the Saguenay region (Québec). The flood deposits covered the indigenous sediments and buried the benthic community. The objective of this work is to determine the best chemical and biological indicators of the changes that occurred in the benthic habitat of the fjord. The new sediment layer is poor in organic matter but rich in detrital carbonates and contains low mercury and lead concentrations reflecting the absence of industrial contamination. Ongoing geological processes leading to the re-establishment of steady-state conditions were evidenced by measurements of porosity, redox potential (Eh), and salinity of interstitial waters taken a few weeks and a few months after the events. The meiofauna were severely affected with a massive reduction of the average density of organisms relative to values recorded before the flood, but recolonisation was already in progress 1 year after the flood. Important losses of macrobenthic species were recorded in 1997 relative to 1996. However, the abundance and the diversity of the polycheate taxa were higher in 1997, indicating a recolonisation of the new sediment layer by pioneer species.

Introduction

Les changements climatiques qui affectent notre planète induisent à l'échelle régionale des crues et des inondations dont l'amplitude et la fréquence répondent à de faibles perturbations climatiques locales. Celles-ci ne peuvent être prévues par les modèles de circulation globale développés à ce jour pour l'étude du réchauffement planétaire (Knox 1993). La presse internationale rapporte souvent les effets dévastateurs de crues soudaines provoquées par d'intenses dépressions atmosphériques apportant de grandes quantités de pluie sur de brèves périodes. Il est cependant très rare que les conséquences de tels accidents climatiques soient examinées pour les zones estuariennes et côtières recevant les eaux douces et les alluvions générées par ces tempêtes (Nichols 1977). Toutefois une crue éclair survenue dans la région du Saguenay au nord du Québec en juillet 1996 (Yu et al. 1997) a motivé la mise en place d'une vaste étude des effets de cette crue sur le fjord du Saguenay et la baie des Ha! Ha! (fig. 1).

La région du fjord du Saguenay a connu au cours du dernier siècle quelques épisodes catastrophiques dont les traces

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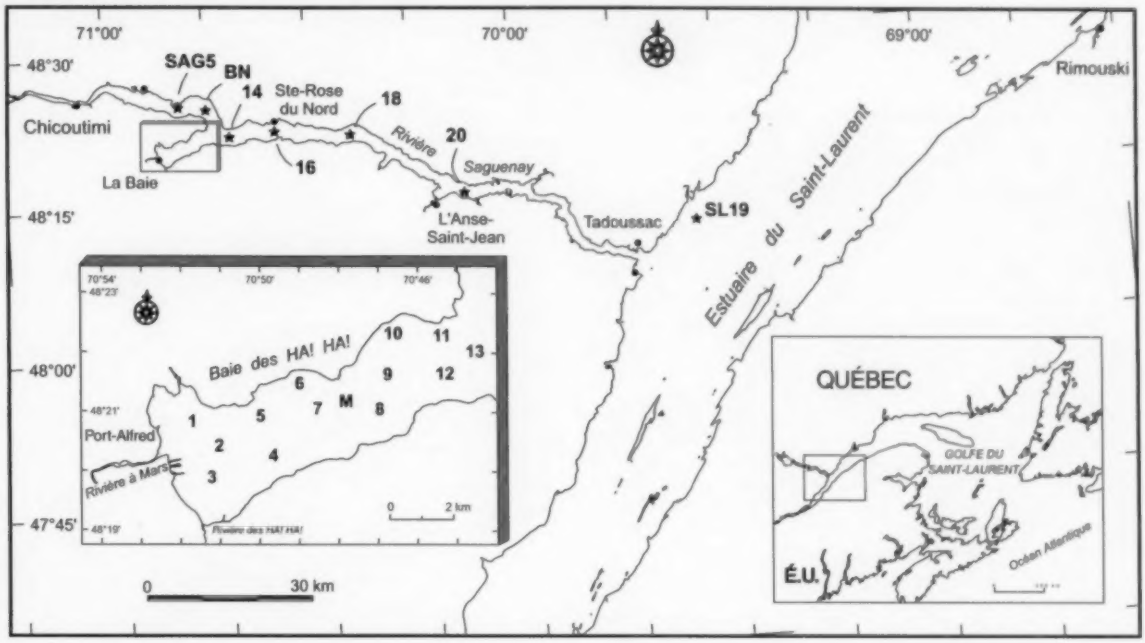
Fig. 1. Localisation des stations d'échantillonnage dans la baie des Ha! Ha! et le fjord du Saguenay lors des expéditions océanographiques de septembre 1996 et août 1997.



demeurent bien visibles dans les sédiments. Une étude détaillée menée à la fin des années 1970 (Schafer et al. 1980; Smith et Schafer 1987) sur des sédiments de la partie amont du fjord a montré la signature vestige d'importantes crues printanières et de glissements de terrain catastrophiques. Par contre, la baie des Ha! Ha!, par sa position en retrait du courant principal du fjord du Saguenay et aussi par l'absence d'apport provenant de rivières importantes en amont n'a pas gardé trace des fluctuations catastrophiques enregistrées dans le bras nord. La géochimie des sédiments du Saguenay a fait l'objet de plusieurs études suite à la découverte au début des années 1970 d'une contamination de ce fjord par le mercure et certains autres métaux (Loring et Bewers 1978) ainsi que la présence des hydrocarbures aromatiques (HAP) (Martel et al. 1987). Par contre, l'écologie benthique du fjord et de la baie des Ha! Ha! a été peu étudiée, et ce n'est que récemment qu'un inventaire exhaustif des invertébrés a été publié par Bossé et al. (1996).

L'ampleur de la crue éclair de juillet 1996 est illustrée par le débit de la rivière Saguenay qui est passé d'environ 1600 à 5500 $m^3 \cdot s^{-1}$ en 48 h (Gilbert 1997). Entre les 19 et 26 juillet le volume d'eau débité par la rivière a atteint $1{,}1 \times 10^9$ m^3, soit le double de son régime normal. Dans la baie des Ha! Ha!, le volume écoulé par la rivière Ha! Ha! et par la rivière à Mars pour la période du 19 au 24 juillet a été estimé à environ 2×10^8 m^3 (Nicolet et al. 1997) ce qui représente plusieurs dizaines de fois le volume normalement débité par ces deux petites rivières en été (8 à 15 $m^3 \cdot s^{-1}$). Il n'existe aucune donnée sur la charge sédimentaire transportée par ces rivières au moment de la crue. Cependant, des travaux sur l'hydrologie dans la vallée de la rivière des Ha! Ha! ont montré que le bilan net des matériaux sédimentaires (gravier, sable et argile) déplacés le long de cette vallée a été de l'ordre de 15 millions de tonnes (Lapointe et al. 1998). Même si une partie de ces matériaux les plus grossiers s'est déposée au niveau du delta de la rivière, plusieurs expéditions océanographiques successives en 1996 et 1997 ont rapporté la présence d'une couche sédimentaire grisâtre d'épaisseur variable s'étendant sur toute la superficie de la baie des Ha! Ha! et dans une partie du bras nord du fjord. La baie des Ha! Ha! devenait ainsi un site privilégié pour une étude des conséquences d'un recouvrement d'un fond marin par de nouveaux sédiments à la fois sur la géochimie et l'écologie benthique.

Dans un premier article sur les effets de la crue éclair (Pelletier et al. 1999), les auteurs ont montré que les meilleurs indicateurs chimiques et biologiques permettant d'évaluer l'ampleur des dommages causés à l'écosystème pélagique de la baie et du fjord étaient l'abondance et la nature chimique du matériel particulaire en suspension ainsi que l'abondance et l'état physiologique du mésozooplancton. L'objectif de ce deuxième article est d'identifier les meilleurs indicateurs chimiques et biologiques qui permettent de caractériser les changements à court terme survenus dans l'habitat benthique de la baie des Ha! Ha! et la zone aval du fjord. Nous décrivons d'abord quelques paramètres chimiques et géochimiques qui semblent les plus pertinents à la caractérisation de la nouvelle couche sédimentaire par rapport à l'ancienne. Nous présentons ensuite les indices permettant de déterminer si la distribution et l'abondance des organismes composant l'écosystème benthique ont été modifiées après l'apport brusque des sédiments. Dans un cadre plus général, cette étude a pour but d'établir quelques points de références spatio-temporels, à la fois pour la géo-

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chimie et l'écologie benthique, qui permettront d'appuyer les travaux qui se poursuivent sur ce site.

Matériel et méthodes

Échantillonnage

L'échantillonnage des sédiments pour les analyses chimiques et biologiques a été mené au cours de trois expéditions océanographiques à bord de l'*Alcide C. Horth* du 18 au 25 août 1996, du 21 au 25 septembre 1996 et du 25 au 29 août 1997. La localisation des stations d'échantillonnage est présentée à la figure 1. Pour les analyses minérales de septembre 1996, les échantillons de surface ont été prélevés immédiatement après la récupération des carottes à l'aide d'une mince spatule de plastique sur une surface de 100 à 200 cm^2 et une profondeur maximale de 5 mm. Toutes les carottes de sédiments ont été immédiatement sous-échantillonnées à l'aide d'une table à découper (Edenborn et al. 1986). Pour chaque tranche de sédiment (de 0,5 à 5 cm selon le cas), la porosité, le contenu en carbone total et en carbone inorganique, le rapport molaire élémentaire C_{org}/N et les teneurs en hydrocarbures (*n*-alcanes) ont été déterminés au laboratoire. Les mesures de salinité ont été réalisées à partir des eaux porales extraites par centrifugation (15 min à environ 2000 × *g*) à l'aide d'un réfractomètre de terrain précis à l'unité près. Les mesures du potentiel redox (Eh) du sédiment ont été effectuées en 1996 et 1997 immédiatement à bord du bateau à l'aide d'un potentiomètre de terrain équipé d'un couple d'électrodes platine–calomel. Lors de l'expédition d'août 1997, des échantillons de sédiment ont été obtenus à la station 13 à l'aide d'un carottier multi-prises. Cette carotte a été découpée en tranches de 1 à 6 cm selon la profondeur, et les échantillons ont été immédiatement congelés à –20°C.

Analyses physiques et chimiques

Au laboratoire, les échantillons de sédiment pour la géochimie organique ont été lyophilisés. La porosité a été estimée à partir de la perte en eau après lyophilisation et en utilisant une valeur de 2,50 $g \cdot cm^{-3}$ pour la densité des sédiments secs et 1,03 $g \cdot cm^{-3}$ pour la densité de l'eau interstitielle dans le fjord du Saguenay (Smith et Walton 1980). Le carbone total et l'azote ont été déterminés par combustion à l'aide d'un analyseur élémentaire calibré avec un étalon d'acétanilide. Le contenu en carbone inorganique des sédiments a été déterminé par titrage coulométrique du CO_2 libéré par l'addition d'une solution HCl 2 N. La reproductibilité de cette mesure est meilleure que ±2%. Le contenu en carbone organique (C_{org}) est obtenu par différence entre le contenu en carbone total et le contenu en carbone inorganique avec une inexactitude cumulative de ±4%. La perte au feu (PAF) a été déterminée par la mesure de la perte de poids du sédiment sec après calcination à 550°C pendant 24 h avec une incertitude de ±5%.

Les analyses de la fraction labile des sédiments ont été effectuées par le Centre de recherche minérale de Québec. Chaque sous-échantillon de 0,5 g a été calciné à 550°C et ensuite attaqué à l'acide nitrique 13% (v/v) à 90°C pendant 60 min. Après refroidissement, la solution a été analysée par spectrométrie d'émission atomique au plasma. Les limites de détection en poids sec sont respectivement : 0,2 $mg \cdot g^{-1}$ pour Al et Ca, 1 $\mu g \cdot g^{-1}$ pour Mn, 2 $\mu g \cdot g^{-1}$ pour Pb et 3 $\mu g \cdot g^{-1}$ pour Zn. Le mercure a été dosé par spectrométrie d'absorption atomique – vapeurs froides. L'échantillon de sédiment (0,5 g) a d'abord été chauffé 15 min à 90°C en présence d'une solution contenant HNO_3 (4 N), $KMnO_4$, HCl concentré et H_2O_2 (30%). La digestion a ensuite été complétée par l'addition de 5 mL de H_2O_2 et chauffage de 15 min à 90°C, suivi de l'addition de 5 mL d'H_2SO_4 et d'un nouveau chauffage de 15 min. La limite de détection pour Hg est de 0,05 $\mu g \cdot g^{-1}$. La variabilité moyenne pour les analyses minérales est de ±10%. Les sédiments étalons MESS-1, BEST-1 et CRM008 ont été utilisés pour valider l'ensemble des analyses minérales. La fraction des hydrocarbures *n*-alcanes a été extraite du sédiment lyophilisé par trappage successif sur des cartouches Sep-pak® préalablement conditionnées avec des solvants de différentes polarités selon la procédure de Gilbert et al. (1998). La quantification a été ensuite effectuée par chromatographie gazeuse couplée à la spectrométrie de masse avec une colonne capillaire SPB-5 de 30 m × 0,25 mm (d.i.). Un étalon interne (*n*-alcane C20) a été ajouté à l'échantillon avant extraction pour la quantification. Le blanc de procédure ne révélait aucune contamination.

Analyse de la faune benthique

Des carottes à boîte ont été prélevées aux stations 2, 7, 9, 13 et 18 en septembre 1996 et août 1997 pour l'étude de la méiofaune. De chaque carotte, trois sous-échantillons ont été prélevés à l'aide d'un petit carottier ayant une section interne de 7,3 cm^2 (Tita et al. 1999). Tous les échantillons ont été fixés et préservés dans le formol 4% (concentration finale). L'extraction de la méiofaune du sédiment a été effectuée par la méthode de la centrifugation avec Ludox-TM (Heip et al. 1985). L'identification taxonomique a été menée au niveau des groupes principaux. La comparaison entre les densités méiofauniques de 1996 et 1997 a été effectuée avec le test de Student. La comparaison entre les densités des différentes stations pour une même année a été effectuée à l'aide d'une analyse de variance.

Seize stations réparties dans la baie des Ha! Ha! (stations 1 à 10 et stations 12 et 13) et dans le bassin profond du fjord du Saguenay (stations 14, 16, 18 et 20) ont été échantillonnées en septembre 1996 pour la détermination de la macrofaune. Pour mesurer l'effet d'une perturbation à grande échelle sur la communauté benthique du Saguenay, il est apparu souhaitable d'adopter par la suite une stratégie d'échantillonnage offrant une plus grande couverture spatiale, impliquant une réduction du nombre d'échantillons par station tout en préservant la précision de l'étude (Cuff et Coleman 1979). En août 1997, le nombre de stations ré-échantillonnées avec le carottier à boîte a été limité à sept dans la baie et le fjord (stations 2, 7, 9, 13, 14 et 16) avec un seul prélèvement par station. La variabilité intra-station a été déterminée en effectuant trois prélèvements à l'aide d'une benne Van Veen. Chaque échantillon de sédiment a été tamisé à l'aide de deux tamis superposés ayant respectivement un vide de mailles de 1 cm et de 1 mm. Les refus de tamis ont ensuite été fixés dans une solution de formaldéhyde (4% concentration finale) tamponnée au borax et colorés au rose Bengale. Au laboratoire, les spécimens ont été triés, dénombrés et identifiés jusqu'à l'espèce dans la mesure du possible.

Résultats et discussion

Les événements de juillet 1996 fournissent une opportunité de documenter un phénomène naturel ayant perturbé l'environnement sédimentaire d'un milieu côtier sur une superficie de plus de 20 km^2. Les effets d'un glissement de terrain sur une communauté benthique médiolittorale peu profonde du golfe Saint-Laurent a déjà fait l'objet d'une étude (Desrosiers et al. 1984). Toutefois, il n'existe à notre connaissance aucun cas documenté sur les effets d'un apport sédimentaire énorme dans un milieu côtier profond (>100 m) comme celui de la baie des Ha! Ha!. L'approche pluridisciplinaire proposée dans cette étude est basée sur l'hypothèse qu'une description adéquate du nouveau milieu sédimentaire par quelques indicateurs chimiques et géochimiques correctement choisis permettra une meilleure compréhension les dommages instantanés causés à l'habitat benthique pour ensuite fournir des indications chimiques et géochimiques sur la consolidation de la nouvelle couche sé-

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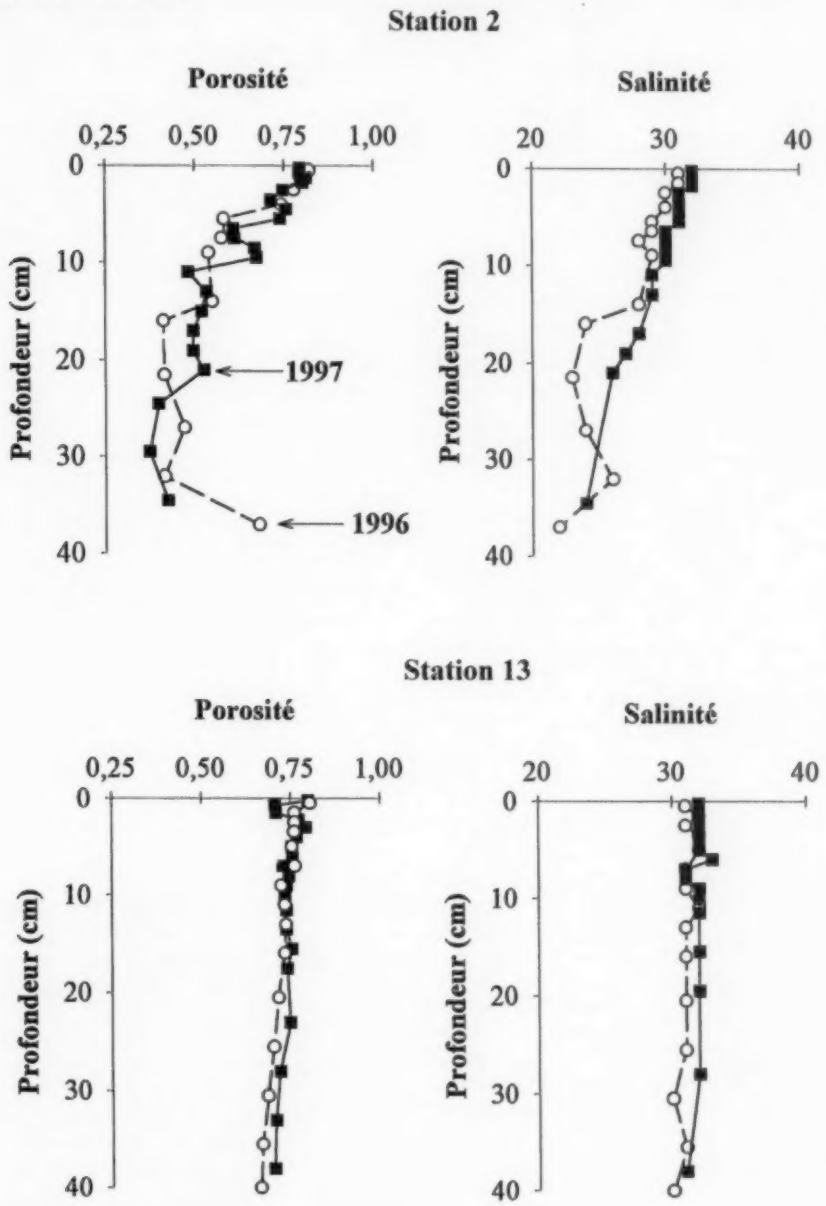
Fig. 2. Distribution des valeurs de porosité et de salinité dans les sédiments des stations 2 et 13 de la baie des Ha! Ha! échantillonnés en septembre 1996 (○) et août 1997 (■).



dimentaire, cette dernière étant essentielle à la recolonisation du milieu dévasté.

Les indicateurs chimiques et géochimiques

Porosité, salinité et Eh

Les profils de porosité de la colonne sédimentaire sont présentés à la figure 2 pour les stations 2 et 13 échantillonnées en septembre 1996 et août 1997. La station 2 montre une même tendance générale pour les 2 années avec des valeurs caractéristiques d'un contenu en eau important en surface (0,82 et 0,79 respectivement pour 1996 et 1997), suivies d'une diminution importante dans les 15 premiers cm pour atteindre des valeurs minimales de 0,41 et 0,53 respectivement. De plus, la carotte de septembre 1996 montre une augmentation rapide en profondeur pour atteindre une valeur proche (avec 0,73 à 38 cm) de celle observée en surface, situant la présence probable de l'interface nouveau–ancien sédiment à cette profondeur. Les porosités de la station 13, située à 10 km en aval de l'embouchure de la rivière des Ha!

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Tableau 1. Données granulométriques et analyse de la fraction labile des sédiments de surface (0–5 mm) échantillonnés en septembre 1996 dans la baie des Ha! Ha! et le bassin profond du fjord du Saguenay.

Station	PAF[a] (%)	Argile (%)	Silt (%)	Sable (%)	Al ($mg \cdot g^{-1}$)	Hg ($\mu g \cdot g^{-1}$)	Pb ($\mu g \cdot g^{-1}$)	Zn ($\mu g \cdot g^{-1}$)	Ca/Al	Mn/Al × 10^3
2	6,6	13,2	75,0	11,8	12,2	0,06	7	48	0,90	25,4
3	7,8	21,4	73,3	5,3	16,1	<0,06	7	60	1,18	23,6
4	7,9	19,3	71,1	9,6	16,1	<0,05	6	59	1,04	24,2
5	7,7	19,9	71,4	8,7	15,7	<0,05	5	59	1,06	25,5
6	7,5	18,6	76,4	5,0	15,7	<0,05	7	61	1,20	22,3
7	7,9	18,1	72,9	8,9	15,1	<0,05	7	55	0,95	26,5
8	7,8	19,3	72,9	8,0	15,5	<0,05	5	62	1,06	27,7
9	7,4	18,4	75,1	6,5	13,9	<0,05	5	54	1,22	24,5
10	7,5	14,0	77,3	9,7	14,4	<0,05	6	58	1,24	22,9
12	7,4	17,6	70,6	12,2	15,3	<0,05	6	56	1,02	28,1
13	7,0	16,4	64,2	19,4	14,9	<0,05	7	56	1,06	29,5
14	6,7	18,4	68,8	12,8	16,2	<0,05	6	58	0,88	27,2
16	7,4	17,8	64,5	17,7	15,3	<0,05	5	58	0,93	22,8
18	11,2	9,2	67,9	22,9	15,4	0,34	19	62	0,40	63,0
20	11,1	7,7	66,9	25,0	14,0	0,17	13	61	0,39	250,0

[a]PAF, perte au feu.

Ha!, présentent des profils normaux sans gradients importants en 1996 et en 1997. Les changements brusques de porosité à la station 2 confirment la présence de plusieurs lits sableux successifs observés lors du découpage des carottes. La présence de sédiments grossiers dans la nouvelle couche sédimentaire peut s'expliquer par la proximité des rivières à Mars et des Ha! Ha! dont les débits ont été multipliés par 10 à 50 fois pendant la crue éclair (Lapointe et al. 1998).

Les données de la salinité des eaux porales à la station 2 peuvent être associées aux données de porosité en particulier pour la carotte prélevée en septembre 1996 (fig. 2). La salinité chute de 31 en surface à 25 à 46 cm et montre deux minimums aux profondeurs de 20 et 37 cm. Ces anomalies coïncident approximativement avec celles observées dans le profil de porosité de la même station. La présence de ces faibles salinités dans les sédiments de la station 2 ne peut s'expliquer que par le dépôt de suspensions sédimentaires enrichies en eau douce. Ces suspensions ont pu se former lorsque le débit de la rivière des Ha! Ha! est passé de 8 à 1100 $m^3 \cdot s^{-1}$. La présence de telles anomalies de salinité ne peut s'expliquer que par l'importance et la rapidité de l'événement sédimentaire emprisonnant alors les eaux douces dans le sédiments. Ces anomalies correspondent à des salinités intermédiaires entre celles des eaux douces transportées et celle des eaux salées du fond de la baie. En août 1997, la distribution de la salinité montre encore de faibles anomalies dénotant un retour progressif aux conditions initiales par un mécanisme actif de la diffusion moléculaire des sels dissous. La station 13 présente des distributions de la salinité classiques avec des valeurs relativement constantes en 1996 (31 ± 1; n = 16) et en 1997 (32 ± 1; n = 17) confirmant que la station semble avoir été peu perturbée par la crue éclair.

Le potentiel d'oxydoréduction (Eh) peut fournir une information qualitative des changements des conditions redox rencontrées dans la colonne sédimentaire. Le profil Eh déterminé à la station M en 1996 (résultats non montrés) présente une nette décroissance les valeurs avec la profondeur passant de +132 mV en surface à –128 mV à 19,5 cm. Les Eh mesurés à la même station en 1997 sont nettement plus faibles en surface (+43 mV) et atteignent –316 mV à 20 cm. Des valeurs systématiquement plus faibles et un gradient plus marqué pour les sédiments en 1997 indiquent une évolution temporelle rapide de la nouvelle couche vers les conditions réductrices normalement observées dans cette région (Gouteux 1996).

La comparaison des indices physico-chimiques de porosité, de salinité des eaux porales et de Eh entre les stations 2 et 13 démontre leur utilité comme descripteurs de la perturbation de la colonne sédimentaire et comme indicateurs primaires de l'évolution de la couche nouvellement déposée par la crue éclair. Par contre, ces indicateurs semblent peu efficaces pour situer précisément l'emplacement de l'interface ancien–nouveau sédiment.

Granulométrie et fraction minérale labile

Les données de granulométrie des sédiments de surface échantillonnés dans la baie des Ha! Ha! et le bassin profond du fjord en septembre 1996 sont présentées au tableau 1. L'ensemble des résultats montre une forte similitude granulométrique des sédiments de surface échantillonnés non seulement dans toute la baie des Ha! Ha! mais aussi plus loin dans le fjord jusqu'à la station 16. Ces sédiments d'aspect gris et fluide possédaient une teneur moyenne en eau de 57 ± 3% et une granulométrie moyenne de 18 ± 2% en argile, 72 ± 4% en silt et 10 ± 4% en sable. Les sédiments à la surface des stations 18 et 20 avaient un contenu en argile plus faible que les stations en amont. Les mesures de perte au feu fournissent une indication sommaire sur les teneurs en matière organique totale, en carbonates et autres matières volatiles (incluant l'eau inter-couche des argiles). De nouveau, les valeurs sont très voisines pour les stations 2 à 16 (7,4 ± 0,4%), alors que les stations non affectées du fjord profond montrent des valeurs significativement plus élevées (11,1 ± 0,1%).

Les résultats de l'analyse de la fraction minérale labile des sédiments par une attaque acide partielle (tableau 1) montrent d'abord les faibles concentrations de mercure et de plomb dans les sédiments superficiels des stations 2 à 16 par

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Tableau 2. Données granulométriques et analyse de la fraction labile des sédiments d'une carotte prélevée à la station 13 dans la baie des Ha! Ha! en août 1997.

Profondeur (cm)	PAF (%)	Argile (%)	Silt (%)	Sable (%)	Al ($mg \cdot g^{-1}$)	Hg ($\mu g \cdot g^{-1}$)	Pb ($\mu g \cdot g^{-1}$)	Zn ($\mu g \cdot g^{-1}$)	Ca/Al	Mn/Al × 10^3
0–1	10,1	14,3	81,9	3,8	15,5	0,06	11	83	0,56	90,3
1–2	7,4	16,9	80,9	2,2	15,0	0,05	12	85	0,95	37,3
2–3	7,5	15,0	80,8	4,2	13,9	0,05	9	79	1,14	23,7
3–4	4,6	11,9	80,7	7,4	10,1	<0,05	8	60	1,55	21,8
4–5	5,3	13,4	84,4	2,2	11,1	0,06	8	65	1,60	20,7
5–6	3,6	9,0	84,6	6,4	7,4	<0,05	7	54	2,23	23,0
6–8	4,7	13,9	80,5	5,6	9,5	0,05	9	58	1,80	22,1
8–10	5,1	7,5	81,5	11,0	9,1	0,12	8	57	1,75	22,0
10–12	4,5	5,7	64,5	29,8	5,5	0,10	6	43	2,47	27,0
12–14	4,7	8,9	68,9	22,2	9,6	0,18	13	60	1,01	28,1
14–18	7,8	14,5	79,4	6,1	15,3	0,54	25	87	0,46	21,6
18–22	8,1	17,1	75,3	7,6	17,3	0,70	33	96	0,43	18,5
22–26	7,9	16,0	81,4	2,6	17,9	1,10	42	110	0,46	17,3

rapport aux stations 18 et 20 qui demeurent contaminées par le mercure à des niveaux toutefois inférieurs à ceux observés aux mêmes stations en 1986 (Pelletier et Canuel 1988). En supposant que les teneurs en aluminium labile sont représentatives de la composition géochimique du sédiment total (fractions labile et réfractaire), on peut mettre en évidence l'enrichissement ou l'appauvrissement des nouveaux sédiments en éléments majeurs et en métaux traces en établissant le rapport à l'aluminium labile. Même si une certaine pollution par l'aluminium pourrait avoir été générée par l'industrie locale, le rapport métal/Al risque peu d'être affecté, puisque les concentrations en Al sont de 1 000 à 10 000 fois plus grandes que celles des métaux traces. Le rapport Mn/Al, un indicateur de la remobilisation diagénétique du manganèse dans les sédiments anoxiques vers la couche oxique superficielle, est faible et constant pour toutes les stations 2 à 16, indiquant que la diagenèse primaire pour ces éléments n'avait pas encore pris place de façon mesurable 2 mois après l'apport massif des sédiments au fjord. Les sédiments déjà en place des stations 18 et 20 montrent clairement l'excès de manganèse dans la couche de surface non modifiée par les nouveaux sédiments. Le calcium labile est un élément majeur que l'on peut aussi comparer à l'aluminium. Le rapport Ca/Al nous apprend que les nouveaux sédiments (terres agricoles, sols forestiers et anciens dépôts marins) sont deux à trois fois plus riches en calcium que les sédiments du fjord profond (station 20). Cette différence tient à la présence élevée de carbonate de calcium dans les nouveaux sédiments.

Les données granulométriques et l'analyse de la fraction labile des sédiments provenant d'une carotte échantillonnée à la station 13 en août 1997 sont présentées au tableau 2. Les sédiments de cette carotte (différente de celle étudiée pour la porosité et la salinité à la figure 2 mais provenant du même secteur géographique) présentent plusieurs caractéristiques remarquables. La granulométrie de la couche superficielle (0–1 cm) est déjà bien différente des sédiments prélevés au même endroit en 1996, et on note la présence d'un lit sableux entre 10 et 14 cm où la proportion en sable atteint presque 30% et l'argile est réduite à ~6%.

La concentration des métaux labiles dans cette carotte met en évidence la présence d'une couche sédimentaire d'environ 12 cm d'épaisseur dont les caractéristiques géochimiques diffèrent sans équivoque des sédiments sous-jacents. La contamination des anciens sédiments de la baie par le mercure et le plomb (Barbeau et al. 1981) apparaît clairement dans les tranches à plus de 14 cm de profondeur. Le zinc, considéré aussi comme un polluant des sédiments du Saguenay, fournit un signal moins clair. La concentration du mercure total mesurée dans un échantillon de surface prélevé à la hauteur de la station 13 en 1986 était de 0,66 $mg \cdot g^{-1}$ (poids sec) (Pelletier et Canuel 1988); une valeur très voisine de celle retrouvée dans la carotte de 1997 à la profondeur 14–18 cm (0,54 $mg \cdot g^{-1}$). Le rapport Mn/Al montre que la remobilisation du manganèse est bien amorcée avec un facteur d'enrichissement dépassant en surface quatre fois les valeurs en profondeur. Le rapport Ca/Al est plus élevé pour les nouveaux sédiments que pour les anciens. Ce rapport apparaît comme un indicateur de l'épaisseur de la nouvelle couche déposée à la station 13, bien qu'il y ait dissolution progressive du $CaCO_3$ et une perte en calcium dans la phase solide (résultats non montrés).

L'ensemble des analyses minérales permet de caractériser clairement la nouvelle couche sédimentaire comme étant pauvre en carbone total mais riche en calcium. Elle ne contient ni mercure, ni plomb anthropiques. Un peu plus d'1 an après le dépôt, le manganèse a nettement amorcé sa remobilisation diagénétique à la faveur du développement d'un milieu de plus en plus réducteur montré par les données de Eh du centre de la baie.

La matière organique

Le carbone organique (% C_{org}) et le rapport C_{org}/N ont été déterminés pour les stations 2 et 13 en septembre 1996 (fig. 3). Les profils verticaux des ces indices sont stables pour la station 13, alors qu'ils apparaissent fortement perturbés à la station 2. Les données de la station 13 correspondent aux résultats déjà publiés pour cette région du Saguenay (Mucci et Edenborn 1992) laissant supposer une colonne sédimentaire peu ou pas influencée par la crue. Les profils de la station 2 comportent de fortes anomalies de distribution. Le % C_{org} est anormalement faible (~1%) dans les premiers 20 cm et montre ensuite un pic majeur (8%) à 40 cm. Le rapport C_{org}/N tend à être plus élevé sur toute la

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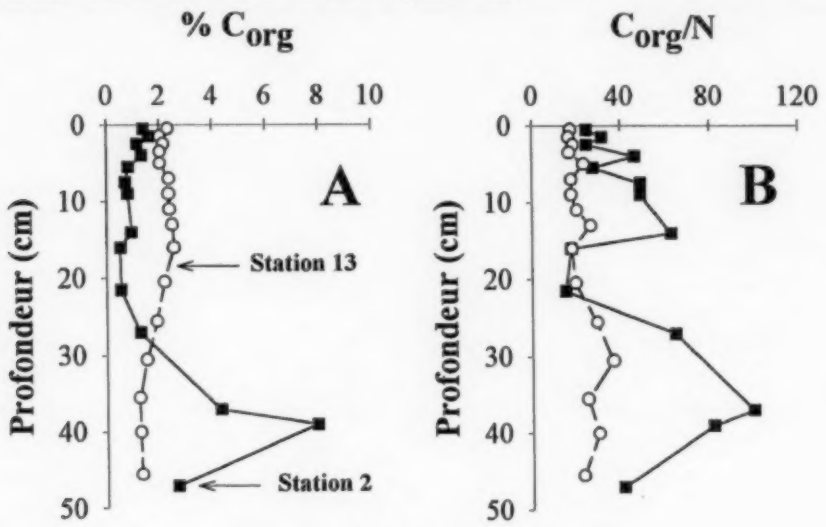
Fig. 3. Distribution des teneurs en carbone organique (% C_{org}) et du rapport C/N en fonction de la profondeur dans les sédiments de la baie des Ha! Ha! échantillonnés aux stations 2 (○) et 13 (■) en septembre 1996.



colonne sédimentaire avec de brusques fluctuations dans les premiers 20 cm et un maximum vers 40 cm. Les valeurs élevées de ces indicateurs en profondeur de la station 2 peuvent être associées à un enfouissement rapide de matériel organique fraîchement déposé à l'interface eau–sédiment originale. On peut aussi supposer une contribution significative de l'endofaune sédentaire vivant à l'interface eau–sédiment qui a été piégée et engloutie au cours du rapide dépôt de matériel sédimentaire. Des rapports C_{org}/N supérieurs à 50 n'ont jamais été observés dans cette région du fjord, à l'exception d'anomalies constatées dans la couche sédimentaire laissée par le glissement de terrain de Saint-Jean Vianney (Mucci et Edenborn 1992). Les importants C_{org}/N retrouvés dans le matériel déposé à la station 2 reflètent une composition de matériaux fortement dégradés et pauvres en azote, correspondant probablement aux dépôts glaciaires érodés des berges des rivières à Mars et Ha! Ha!.

La distribution des hydrocarbures aliphatiques saturés (*n*-alcanes) permet de compléter la caractérisation de la nature de la matière organique présente dans les sédiments. L'utilisation d'indices propres aux différentes fractions de *n*-alcanes permet de reconnaître une possible contamination pétrolière. La concentration en *n*-alcanes totaux aux stations 2 et 13 variaient entre 2,62 et 36,88 $\mu g \cdot g^{-1}$ (poids sec). Les valeurs étaient plus élevées en surface qu'en profondeur sans que des différences majeures apparaissent entre les deux stations. Les alcanes les plus abondants étaient C27 > C29 > C23 (plantes terrestres). Toutefois, le C17 dominait à la surface de la station 2, indiquant la présence ponctuelle de matière organique autochtone fraîchement déposée via la colonne d'eau rapidement redevenue productive (Pelletier et al. 1999). Le rapport FPM/HPM (Σ alcanes < C20/Σ alcanes > C20) était égal à 3,0 en surface de la station 2, confirmant l'apport de carbone organique frais provenant des algues phytoplanctoniques (Colombo et al. 1989).

L'index préférentiel de carbone (ICP = 2(C27 + C29)/(C26 + 2C28 + C30) fournit une valeur proche de 1 pour les hydrocarbures pétrogéniques et comprise entre 3 et 6 pour les plantes vasculaires et les sédiments non contaminés (Colombo et al. 1989). Les ICP mesurés aux stations 2 et 13 se situent tous entre 1,87 et 3,0 permettant d'écarter l'idée d'une contamination pétrolière importante et ubiquiste des sédiments déposés pendant la crue dans la baie. Toutefois, des taches irridescentes généralement associées aux hydrocarbures fossiles ont été notées à quelques reprises à la surface de carottes prélevées dans la baie des Ha! Ha! entre les stations 2 et 13, laissant croire qu'une certaine contamination a pu être introduite dans la baie par la crue éclair.

L'ensemble des indices granulométriques et chimiques permettent de bien caractériser la nouvelle couche sédimentaire dans la baie des Ha! Ha! et en aval dans le fjord. Ces indicateurs permettent de montrer l'évolution temporelle de la géochimie et d'y associer un rétablissement de conditions favorables à la vie benthique (consolidation du sédiment et qualité de la matière organique). À partir de ces paramètres de base sur la qualité du milieu, il importait pour nous de déterminer les meilleurs indices des dommages causés à la faune benthique, d'une part, et d'évaluer les indices d'un rétablissement de l'écosystème, d'autre part.

Caractérisation de la faune benthique

Les communautés benthiques sont classiquement utilisées en tant qu'indicateurs biologiques des effets des perturbations de l'environnement (Pearson et Rosenberg 1978; Hall 1994). Elles sont constituées d'une grande majorité d'espèces sédentaires qui sont autant d'agents intégrateurs des effets temporels des divers stress environnementaux, qu'ils soient d'origine naturelle ou anthropique. De par sa position dans le milieu, le benthos est susceptible d'intégrer les variations et les perturbations qui affectent à la fois la colonne d'eau et la colonne sédimentaire. Nos travaux ont porté à la fois sur la méiofaune constituée par les espèces de taille entre 63 µm et 1 mm et sur la macrofaune formée des espèces de taille supérieure à 1 mm.

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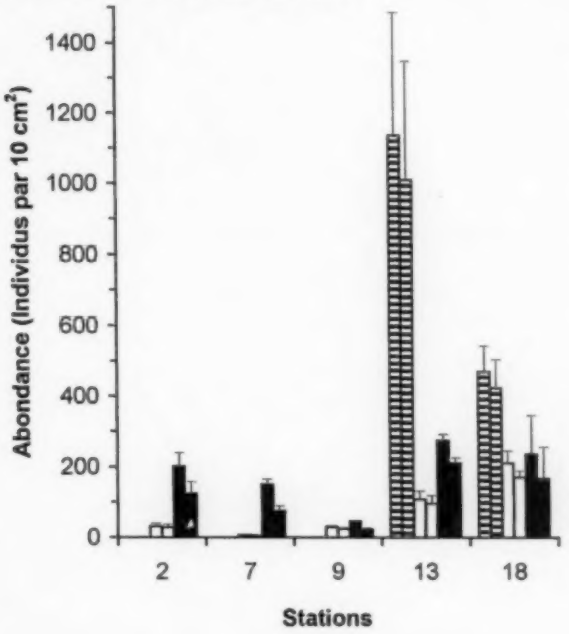
Fig. 4. Densités moyennes de la méiofaune avant et après la crue éclair de juillet 1996. Pour chacune des périodes, les densités moyennes de la méiofaune (barre de droite) et des nématodes (barre de gauche) sont indiqués en nombre d'individus par unité de surface (10 cm^2). Barres à rayure, mai 1996; barres blanches, septembre 1996; barres noires, août 1997.



Les indices de la méiofaune

Il n'existe aucune donnée publiée sur l'abondance et la diversité de la méiofaune benthique de la baie des Ha! Ha! et du fjord du Saguenay. Cependant, le contenu en méiofaune des échantillons obtenus en mai 1996 à deux sites d'échantillonnage correspondant précisément aux stations 13 et 18 ont permis d'établir quelques points de références utiles à l'évaluation de l'impact de la crue, puisque les indices chimiques montrent que ces stations ont été peu affectées par la crue.

En septembre 1996, les densités moyennes de la méiofaune totale dans la baie des Ha! Ha! (stations 2, 7 et 9) étaient réduites de plus de 97% par rapport à celle enregistrée à la station 13 au printemps de la même année (fig. 4). Cette réduction est aussi significative ($P < 0,05$) à la station 13, avant et après la crue éclair, mais un peu moins marquée. En septembre 1996, les densités sont significativement différentes entre la station 13 et les stations 2, 7 et 9 (test NSK, $P < 0,05$). La station 18 semble aussi avoir été affectée par la crue avec une réduction de 55% de ses effectifs entre le printemps et le mois de septembre 1996, contrairement à ce que les indices chimiques laissaient voir. Cette réduction est principalement causée par une diminution du nombre de nématodes ($P < 0,05$) et par l'absence de nauplii. Ces dernières étaient absentes dans toutes les stations en septembre 1996 (tableau 3). Deux taxons, les kinorhynches et les ostracodes, étaient présents seulement à la station 18 avec de très faibles abondances en 1996 (résultats non montrés). Dans la zone la plus affectée par la crue selon les indices physico-chimiques (stations 2, 7, 9 et 13), les densités de nématodes et de copépodes ont été réduites de manière significative ($P < 0,05$). Enfin, concernant la méofaune temporaire, les polychètes ont subi une réduction significative de leurs effectifs aux stations 2, 7 et 9 mais non à la station 13 ($P = 0,096$ et $P = 0,804$, respectivement).

Un an après la crue éclair, les effectifs de la méiofaune totale ont augmenté de manière significative aux stations 2, 7, 9 et 13 ($P < 0,05$) mais demeurent encore réduits par rapport à ceux enregistrés avant la crue à ces mêmes stations (fig. 4). Une différence significative des densités de la méiofaune totale à ces stations est observée entre la station 9 et les stations 2, 7 et 13 (test SNK, $P < 0,05$). Les effectifs des nématodes et des copépodes ont augmenté aux stations 2, 7 et 13 ($P < 0,05$) mais non à la station 9 (tableau 3). Les densités des polychètes ont également augmenté aux stations 2 et 7 ($P < 0,05$). Pendant cette même période, les densités de la méiofaune totale et des différents taxons n'ont pas changé à la station 18. Enfin, il importe de souligner que les nauplii ont réapparu à toutes les stations en 1997 (tableau 3).

Il nous apparaît certain que l'apport brusque et massif de sédiment a sévèrement affecté la méiofaune, à la fois en termes de diversité et d'abondance, non seulement dans la baie des Ha! Ha! mais également dans la zone plus en aval dans le fjord. La composante méiofaunique de la station 18 semble aussi avoir été affectée malgré l'absence d'indices chimiques de perturbation dans le sédiment. Cependant les travaux sur la colonne d'eau (Pelletier et al. 1999) ont montré que l'abondance et la composition du matériel particulaire en suspension dans la couche profonde des eaux du fjord ont été modifiées par la crue durant plus d'une année, ce qui pourrait suffire à induire des conditions défavorables à certaines espèces de la méiofaune. Un an après le recouvrement, les densités de la méiofaune totale ainsi que le nombre des taxons présents ont augmenté significativement aux stations situées dans la zone la plus affectée (à l'exception de la station 9), ce qui suggère une recolonisation rapide de la nouvelle couche sédimentaire. La détermination de la méiofaune constitue un indicateur puissant et précis des dommages instantanés causés à l'écosystème benthique dans la zone perturbée.

Les indices de la macrofaune

L'abondance en organismes macrobenthiques rencontrés à chacune des stations échantillonnées en septembre 1996 dans la baie des Ha! Ha! est présentée à la figure 5A. Les stations 2 et 3 sont caractérisées par l'absence complète d'organismes macrobenthiques. Pour les autres stations, les densités ne dépassaient jamais 43 individus par 600 cm^2 (station 12). La station 13 est caractérisée par une très faible densité en organismes macrobenthiques, alors que d'autres stations (4, 7, 8 et 9) situées nettement à l'intérieur de la baie montrent des densités bien supérieures, même si ces stations ont normalement été plus affectées que la station 13 comme le montrent les indices chimiques. La distribution des différents taxons à chacune des stations (en % du total) est présentée à la figure 5B. Les annélides polychètes étaient les mieux représentés, puisqu'ils occupaient 13 des 16 stations échantillonnées. En terme d'effectifs, ils dominent même dans 10 de ces stations. Le groupe des mollusques, représenté par des bivalves du genre *Macoma*, est signalé à

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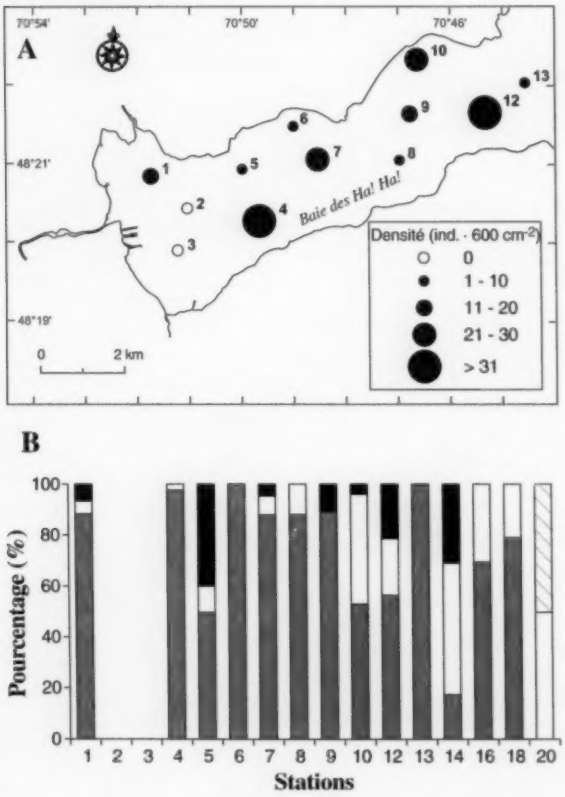
Tableau 3. Distribution (individus par 10 cm^2) des différents groupes méiofauniques échantillonnés en septembre 1996 et août 1997.

	Nématodes		Copépodes		Nauplii		Turbellariés		Polychètes		Isopodes		Total	
Stations[a]	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
2	29±15	125±57	2±1	29±17	—	28±5	—	8±5	1+1	11±10	—	—	31±16	201±68
	(94)	(62)	(4)	(14)		(14)		(4)	(2)	(6)			(100)	(100)
7	6±4	76±24	1+1	21±6	—	35±5	—	8±5	1+2	9±6	—	—	7±5	150±27
	(80)	(51)	(7)	(14)		(23)		(6)	(13)	(6)			(100)	(100)
9	25±7	16±9	4±3	11±4	—	8±2	—	—	2±2	10±2	1±1	—	31±6	45±2
	(81)	(36)	(12)	(24)		(18)			(6)	(21)	(2)		(100)	(100)
13	97±42	211±28	5±3	18±1	—	16±3	1±1	8±2	6±4	18±9	1±1	2±1	109±42	274±32
	(89)	(77)	(5)	(7)		(6)	(1)	(3)	(5)	(7)	(<1)	(1)	(100)	(100)
18	171±35	168±156	18±25	26±16	—	24±12	1±2	7±5	17±7	8±3	—	—	211±63	237±189
	(81)	(71)	(9)	(11)		(10)	(1)	(3)	(8)	(3)			(100)	(100)

[a]Les abondances relatives sont données en pourcentage, entre parenthèses, pour chaque station.

Fig. 5. Distribution spatiale de la densité des individus de la macrofaune (A) et représentation en pourcentage des taxons (B) présents aux stations échantillonnés en septembre 1996 (barres blanches, ollusques; grises, polychètes; noires, crustacés; rayées, échinodermes).



plusieurs stations mais ne dominait qu'à la station 14. Enfin, le groupe des échinodermes n'était présent qu'à la station 20.

La distribution des différentes familles de polychètes pour les 16 stations est indiquée au tableau 4. Sur un total de neuf familles, les plus fréquemment rencontrées sont les Cirratulidae (genre *Chaetozone*), les Lumbrineridae (genre *Lumbrineris*), les Maldanidae (*Maldane sarsi*), les Spionidae (essentiellement dominés par *Lanice cirrata* et *Prionospio steenstrupi*), et les Orbiniidae (genre *Leitoscoloplos*). Une analyse par groupements à liens complets met en évidence le regroupement de certaines stations (fig. 6). Ainsi, les stations 2, 3, 5 et 6, situées dans la partie amont de la baie et dans le prolongement de la rivière Ha! Ha!, forment un groupe bien distinct de celui composé des stations 7, 9, 10 et 12 localisées dans la moitié aval de la baie. Le premier groupe a été plus fortement touché par les apports de sédiment, tandis que le second groupe est formé de stations probablement moins perturbées parce que mieux protégées du flux initial de la boue et des débris apportés par la crue.

Sept stations ont fait l'objet d'un deuxième échantillonnage en août 1997 (les stations 2, 7, 9 et 13 dans la baie et les stations 14, 16 et 18 dans le bassin profond). La comparaison de la présence–absence entre les réplicats échantillonnés avec la benne Van Veen ne montre pas de différence pour le taxon dominant des polychètes. Par contre, les abondances en organismes macrobenthiques de 1997 sont comparées à celles de 1996 à la figure 7. Fait marquant, les densités observées en 1997 aux stations situées dans la baie (stations 7 et 9) sont nettement inférieures à celles enregistrées en 1996 aux mêmes stations (station 2 exceptée). Cependant, l'abondance en organismes macrobenthiques est nettement plus forte à la station 13 en 1997, comparativement à 1996. La distribution des différentes familles de polychètes en 1997 aux stations 2, 7, 9, 13, 14, 16 et 18 montrent une réduction dramatique des effectifs aux stations 2, 7 et 9 mais une augmentation importante à la station 13. Les Spionidae qui étaient la famille la plus souvent rencontrée dans ces stations en 1996 est supplantée par les Lumbrineridae (genre *Lumbrineris*) en 1997. La station 13 qui n'était occupée que par les Spionidae en 1996 est, en 1997, composée de six familles parmi lesquelles les Ampharetidae (genre *Ampharete*) et les Capitellidae.

Bien que notre stratégie d'échantillonnage ne nous fournisse que des informations fragmentaires sur la structure et la composition des peuplements du macrobenthos, il est possible d'émettre quelques hypothèses sur la restructuration des communautés dans la baie des Ha! Ha!. Les travaux de bionomie benthique sur le fjord du Saguenay sont peu nombreux (Drainville 1970; Bossé et al. 1996). Les densités en organismes macrobenthiques enregistrées dans l'ensemble

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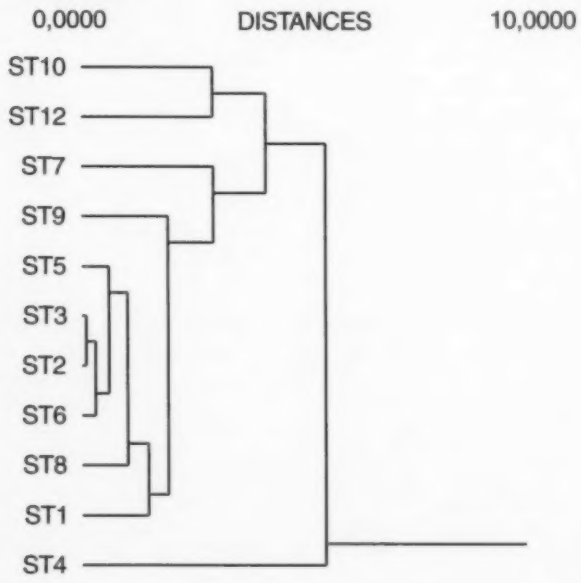
Tableau 4. Distribution (individus par 600 cm^2) des différentes familles de polychètes répertoriées aux stations échantillonnées en 1996.

	Stations[a]												
Familles	1	4	5	6	7	8	9	10	12	13	14	16	18
Cirratulidae	—	30 (78,9)	1 (20)	—	3 (13,6)	2 (28,6)	—	1 (7,1)	3 (12,5)	—	—	2 (22,2)	—
Cossuridae	—	1 (2,6)	—	—	—	—	—	—	—	—	—	—	—
Lumbrineridae	—	5 (13,2)	—	—	—	2 (28,6)	3 (17,6)	8 (57,1)	4 (16,7)	—	4 (80)	6 (66,7)	3 (27,3)
Maldanidae	2 (13,3)	—	—	—	—	—	—	—	—	—	—	—	—
Nephtydae	—	1 (2,6)	1 (20)	—	1 (4,5)	—	—	—	—	—	—	—	—
Ophelidae	—	—	—	—	—	—	4 (23,5)	5 (37,5)	5 (28,5)	—	—	—	3 (27,3)
Orbiniidae	2 (13,3)	—	—	1 (100)	12 (54,4)	3 (42,9)	3 (17,6)	—	8 (33,3)	—	—	—	4 (36,4)
Paraonidae	3 (20)	1	—	—	—	—	—	—	—	—	—	—	—
Spionidae	8 (53,3)	—	1 (20)	—	3 (13,6)	—	4 (23,5)	—	4 (16,7)	4 (100)	1 (20)	1 (11,1)	1 (9,1)
Divers	—	—	2 (40)	—	3 (13,6)	—	3 (17,6)	—	—	—	—	—	—

[a]Les abondances relatives sont données en pourcentage, entre parenthèses, pour chaque station.

Fig. 6. Groupement des stations par la méthode non hiérarchique à liens complets.



des stations (aussi bien en 1996 qu'en 1997) sont plus faibles que celles rapportées par Bossé et al. (1996). L'échantillonnage des stations de la baie des Ha! Ha! en 1997 a révélé une chute drastique des effectifs par rapport à 1996, ce qui suggère une absence ou un échec du recrutement. Par contre, il est important de souligner que la station 13, située à la jonction entre la baie des Ha! Ha! et le bassin profond du fjord, a bénéficié d'une forte augmentation de ses effectifs en 1997 par rapport à 1996. Elle abrite pas moins de six familles dont les représentants sont traditionnellement désignés comme espèces indicatrices d'une contamination organique (Pearson et Rosenberg 1978). L'échantillonnage de 1997 confirme que le taxon des polychètes est bien souvent le mieux représenté, aussi bien en terme d'abondance qu'en terme de diversité spécifique. Il est raisonnable de penser que c'est essentiellement dans ce taxon que nous trouverons les espèces pionnières, caractérisées par des productions larvaires très élevées. Les mollusques rencontrés dans les stations en 1996 et 1997 sont exclusivement représentés par des individus du genre *Macoma* (*M. calcarea*). *Macoma calcarea* peut être considérée comme une espèce indicatrice de contamination du milieu et est aussi décrite comme étant une espèce colonisatrice de zone anoxique (Pearson et Rosenberg 1978).

Conclusions

L'étude des traceurs physico-chimiques et géochimiques dans les sédiments de la baie des Ha! Ha! et de la partie amont du fjord révèle toute l'importance de la perturbation de la colonne sédimentaire suite à la crue de juillet 1996. Plusieurs des paramètres mesurés (porosité, salinité des eaux porales, granulométrie, métaux traces, rapports métal/Al et C/N) se montrent de bons indicateurs des changements chimiques survenus au nouvel interface eau–sédiment et dans la colonne sédimentaire. L'échantillonnage une année après la crue indique des modifications géochimiques rapides de la nouvelle colonne sédimentaire, notammment par la remobilisation diagénétique du manganèse près de la nouvelle interface eau–sédiment. Les indices du carbone organique

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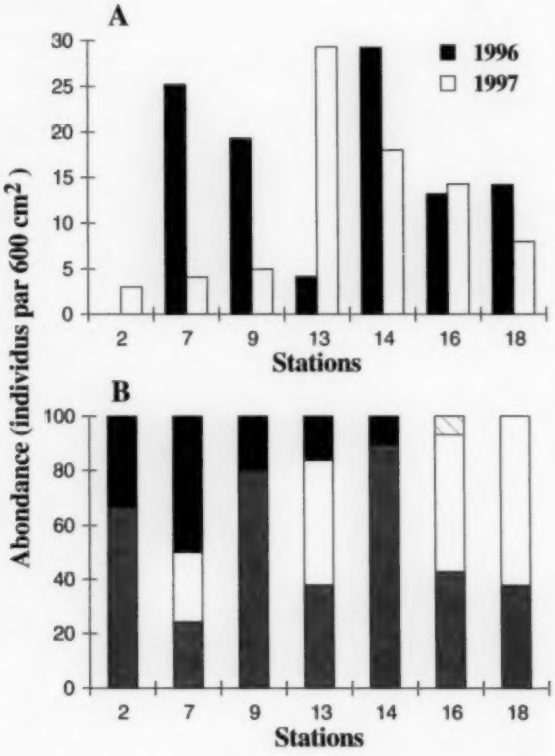
Fig. 7. Nombre total d'individus de la macrofaune (A) et représentation en pourcentage des taxons (B) présents aux stations échantillonnés en août 1997 (barres blanches, mollusques; grises, polychètes; noires, crustacés; rayées, échinodermes).



montrent que la nouvelle couche est relativement pauvre en carbone et azote organique et apparemment peu favorable à la réorganisation de l'écosystème benthique sans l'apport de carbone marin frais. Cependant les indices mesurés pour la méiofaune et la microfaune indiquent un net processus de recolonisation de la nouvelle couche sédimentaire. Malgré les limitations de l'échantillonnage, les indices du macrobenthos sont précieux dans notre recherche de moyens pour évaluer les effets à court terme (quelques mois) de la crue sur la faune benthique. La distribution de la méiofaune et la présence des espèces pionnières de polychètes nous indiquent une recolonisation rapide du site.

Les indicateurs développés lors de cette étude de cas sont d'application générale pour la caractérisation d'un habitat benthique ayant subi une perturbation majeure causée par une crue, un glissement sous-marin ou un tremblement de terre. Notre étude montre que l'approche pluridisciplinaire est essentielle puisque les indices physico-chimiques (moins laborieux et plus rapides à obtenir) fournissent des éléments précis sur l'ampleur des bouleversements sédimentaires, tandis que les indices biologiques (en particulier ceux de la méiofaune et des vers polychètes) nous fournissent la capacité de récupération du milieu perturbé.

L'ensemble des travaux sur le fjord du Saguenay entrepris dans les quelques semaines ayant suivi la crue de juillet 1996 ont permis jusqu'à maintenant d'établir que l'écosystème pélagique avait peu ou pas souffert de cette crue (Pelletier et al. 1999) alors que l'écosystème benthique directement exposé aux apports terrigènes massifs des rivières en crue avait été profondément touché. Dans un contexte plus général, l'ensemble de l'étude a permis de discerner les indicateurs physico-chimiques et biologiques capables de décrire les impacts réels de la crue sur la colonne d'eau et sur l'habitat benthique dans un milieu côtier profond, indicateurs généralement simples à déterminer et qui pourront être utilisés pour la caractérisation d'événements similaires dans d'autres environnements côtiers.

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Individual-based model simulations of a zebra mussel (*Dreissena polymorpha*) induced energy shunt on walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*) populations in Oneida Lake, New York

Edward S. Rutherford, Kenneth A. Rose, Edward L. Mills, John L. Forney, Christine M. Mayer, and Lars G. Rudstam

Abstract: Zebra mussels (*Dreissena polymorpha*) recently invaded North American aquatic ecosystems and are hypothesized to impact lakes by increasing water clarity through filtration and shunting energy from pelagic to benthic pathways (ES effect). We used an individual-based model of Oneida Lake, New York, walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*) populations to simulate ES effects on percids by lowering zooplankton density and production rates and increasing benthos rates. We performed 50-year simulations involving both zooplankton and benthos changes together and each change alone. We also performed simulations to determine robustness of model predictions under different assumed levels of zooplankton and benthos, increased forage fish and mayflies, and walleye stocking. Predicted ES effects were elimination of walleye high-recruitment years, resulting in a 30% reduction in adult walleye abundance. Yellow perch adult abundance increased by 6% due to reduced walleye predation. Neither component (zooplankton or benthos) of ES generated the same total response for yellow perch as both components together. Simulated walleye stocking and increased forage fish density had little effect on model predictions. Increased mayfly densities offset predicted ES effects by buffering predation on juvenile percids. Model predictions of ES impacts on percids generally were consistent with observed changes in Oneida Lake since zebra mussel arrived.

Résumé : La moule zébrée (*Dreissena polymorpha*) a depuis peu envahi les écosystèmes aquatiques d'Amérique du Nord et semble avoir un effet sur les lacs en accroissant la limpidité de l'eau par filtration et en détournant l'énergie des systèmes pélagiques vers les systèmes benthiques (effet ES). Nous avons employé un modèle individualisé des populations de doré (*Stizostedion vitreum*) et de perchaude (*Perca flavescens*) du lac Oneida (New York) pour simuler les effets sur les percidés en abaissant la densité et les taux de production du zooplancton et en accroissant ceux du benthos. Nous avons effectué des simulations de 50 ans portant sur les changements combinés du zooplancton et du benthos, puis sur chacun des changements. Nous avons aussi effectué des simulations visant à déterminer la robustesse des prédictions du modèle à différents niveaux supposés de zooplancton et de benthos, avec une augmentation des poissons fourrage et des éphémères et avec ensemencement de doré jaune. Les effets ES prédits étaient une élimination des années de fort recrutement du doré, ce qui causait une réduction de 30% de l'abondance des dorés adultes. L'abondance des perchaudes adultes a augmenté de 6% par suite de la baisse de la prédation par les dorés. Aucune composante (zooplancton ou benthos) de l'effet ES ne causait à elle seule le même résultat total pour la perchaude que les deux composantes prises ensemble. La simulation de l'ensemencement de doré et de l'accroissement de la densité de poisson fourrage avait peu d'effet sur les prédictions du modèle. L'accroissement de la densité des éphémères annulait les effets ES prédits en compensant la prédation exercée sur les percidés juvéniles. Les prédictions des effets ES sur les percidés selon le modèle correspondaient dans l'ensemble aux changements observés dans le lac Oneida depuis l'arrivée de la moule zébrée.

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Table 1. Mean stage-specific abundances (no./ha) of Oneida Lake yellow perch and walleye from pre-zebra mussel periods (1961–1969, 1970–1978, 1979–1991, 1986–1991) to the post-zebra mussel period (1992–1997).

	Life stage					
Period	Eggs ($\times 10^6$)	18 mm	August	Fall YOY	Fall age 1	Adult
Yellow perch						
1961–1969	2.66	34 180	21 616	2748	136	141*
1970–1978	2.59	95 880	11 910	2050	42	114*
1979–1991	2.63	92 200	7 060	770	27	123*
1986–1991	1.95	41 600	1 530	220	27	90*
1992–1997	1.01	30 583	2 783	154	38	43
Walleye						
1961–1969			166	89	11	36*
1970–1978			62	40	10	28*
1979–1991			36	23	7	32*
1986–1991			14	22	11	37*
1992–1997				9	2	15

Note: Asterisks indicate that the differences in mean abundances between pre-zebra mussel periods and the post-zebra mussel period were significant at the $P < 0.05$ level (ANOVA or Wilcoxon test, $\alpha = 0.05$).

Introduction

Zebra mussels (*Dreissena polymorpha*) were recently introduced into the Great Lakes and are feared to cause major changes to lake ecosystems. They were discovered in Lake St. Clair in 1986, spread rapidly throughout the Great Lakes drainage (Griffiths et al. 1991; Mills et al. 1993), and by 1995 had reached densities in some areas approaching 30 000 mussels/m^2 (Nalepa et al. 1995). Zebra mussels invaded Oneida Lake, New York, in 1991, and by 1995, densities exceeded 26 000/m^2 on hard substrates (Horgan and Mills 1997). In Oneida Lake, as in other waters, zebra mussels proved to be voracious feeders, clearing the water of particulate matter (Mellina et al. 1995; Horgan and Mills 1997). How grazing by zebra mussels will alter food webs and affect important sport fish remains speculative. Although studies have already documented changes in abundance of benthos and zooplankton attributable to zebra mussels (Stewart and Haynes 1994; Karateyev et al. 1997), zebra mussel effects on fish populations are relatively unknown. Studies of zebra mussel effects on fish have been limited to short-term laboratory or controlled field experiments (Jennings 1996; Richardson and Bartsch 1997; Thayer et al. 1997; Mayer 1998). To our knowledge, quantitative predictions of the long-term effects of zebra mussel invasion on fish populations have not yet been attempted.

Zebra mussels are hypothesized to affect aquatic ecosystems by (*i*) shunting energy from pelagic to benthic pathways (energy shunt (ES) hypothesis), (*ii*) improving water clarity and stimulating growth of aquatic macrophytes (Fahnenstiel et al. 1995*a*; Skubinna et al. 1995; Horgan and Mills 1997; Mayer 1998), (*iii*) reducing chlorophyll concentrations (Fahnenstiel et al. 1995*b*), and (*iv*) increasing the frequency and duration of blue-green algal blooms (Griffiths 1993; Nalepa and Fahnenstiel 1995). Initial observations of zebra mussel impacts in the Great Lakes (Griffiths 1993; Stewart and Haynes 1994; Nalepa et al. 1995; Botts et al. 1996), in Oneida Lake (Horgan and Mills 1997; Idrisi 1997; Mayer 1998), and in Europe (Karateyev et al. 1997) have provided supportive evidence for all four hypotheses. More subtle, complex changes in phytoplankton species composition and community structure have also been documented (Fahnenstiel et al. 1995*b*; Mellina et al. 1995; Nalepa and Fahnenstiel 1995).

In Oneida Lake, water clarity and densities of most benthos taxa in shallow water have increased since zebra mussel invased, but no change in algal primary production or cladoceran density has been detected (Horgan and Mills 1997; Idrisi 1997; Mayer 1998). However, the effects of zebra mussels on walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*) populations are not as clear. Zebra mussel effects on percids are confounded by time-lagged effects from irruptions by white perch (*Morone americana*) (1980, 1982–1983) and gizzard shad (*Dorosoma cepedianum*) (1984–1989) during the 1980s (Tables 1 and 2; Fig. 1). Indeed, time series plots of young-of-year (YOY) yellow perch densities indicate continuation of a downward trend in yellow perch abundance and a corresponding increase in yellow perch size, which commenced before zebra mussel invaded (Fig. 2*b*). Recruitment of YOY walleye, which has declined since zebra mussel invaded (Fig. 2*a*), may be attributed to reduced densities of all available forage (yellow perch, white perch, gizzard shad) as well as decreased vulnerability of the longer YOY yellow perch. Confounding effects of time-lagged responses to previous disturbances, interannual environmental variability, and the subtlety of compensatory behavior by walleye and yellow perch in response to disturbances (Forney 1980) underscore the value of a modeling approach for quantifying zebra mussel effects on fishes.

We used 50-year simulations of an individual-based model (IBM) to evaluate the long-term impacts of zebra mussels on walleye and yellow perch populations in Oneida Lake. IBMs are appropriate for examining zebra mussel impacts on walleye and yellow perch because many species interactions are size dependent and are easily represented in IBMs (DeAngelis et al. 1994). We simulated the ES effect by decreasing zooplankton densities and production and increasing benthos densities and production. We hypothesized that the ES effect would cause an ontogenetic bottleneck for yellow perch (Persson and Greenberg 1990); a reduction in zooplankton prey density for YOY yellow perch would

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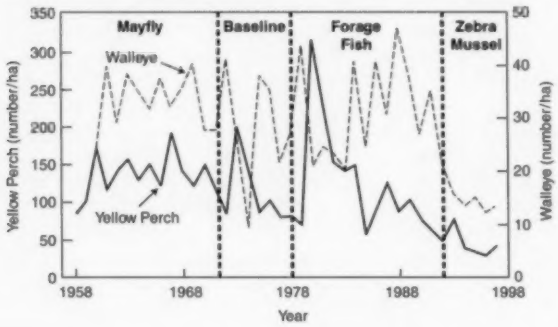
Table 2. Mean stage-specific survival rates (%) of Oneida Lake yellow perch and walleye during pre-zebra mussel periods (1961–1969, 1970–1978, 1979–1991, 1986–1991) and the post-zebra mussel period (1992–1997).

	Life stage				
Period	Egg to 18 mm	18 mm to Aug.	Aug.–Oct.	Oct. YOY to Oct. age 1	18 mm to recruitment
Yellow perch					
1961–1969	1.18	48.86	10.90	6.40*	0.30
1970–1978	3.97	12.42	17.24	3.80*	0.04
1979–1991	3.41	7.66	10.90	16.8	0.03
1986–1991	2.26	3.69	14.35	31.10	0.06
1992–1997	2.98	10.37	7.63	25.00	0.15
Walleye					
1961–1969				12.58	
1970–1978				26.16	
1979–1991				34.75	
1986–1991				44.07	
1992–1997				28.48	

Note: Asterisks indicate that the differences in mean survival rates between pre-zebra mussel periods and the post-zebra mussel period were significant at the $P < 0.05$ level (ANOVA or Wilcoxon test, $\alpha = 0.05$).

Fig. 1. Observed abundances of adult walleyes (≥ age 4) and yellow perch (≥ age 3) in Oneida Lake from 1958 to 1997. The mayfly period, when mayflies were present, extends from 1958 to 1969, the baseline period, when mayflies were absent and forage fish densities were relatively low, extends from 1970 to 1978, the forage fish period, when abundances of white perch or gizzard shad were relatively high, extends from 1979 to 1991, and the zebra mussel period extends from 1992 to the present.



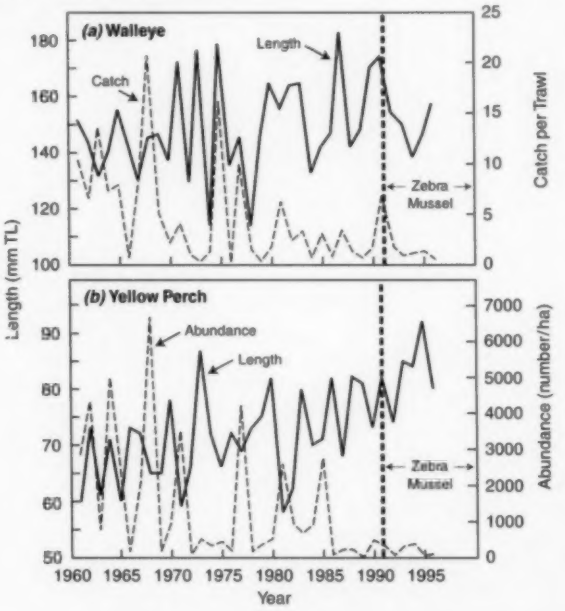
lower YOY growth rate and increase mortality, while an increase in benthos density for yearling and adult yellow perch would increase adult yellow perch growth rate and egg production, further exacerbating YOY pressure on their prey. We hypothesized that zebra mussels would have a negative effect on walleye survival and recruitment because of their negative effect on yellow perch survival. Model simulations were used to evaluate these hypotheses and predictions were compared with available field data from Oneida Lake.

Methods

Oneida Lake site description

Oneida Lake is a large (207 km^2) productive lake in central New York State with a mean depth of 6.8 m and a maximum depth of 16.8 m. Prevailing westerly winds and wind-driven currents generally maintain well-mixed isothermal conditions throughout the

Fig. 2. Trends in mean length and abundance of YOY (*a*) walleye and (*b*) yellow perch estimated from the catch in trawls during October in pre- (1961–1991) and post-zebra mussel years (1992–1997) in Oneida Lake.



summer, except for brief periods of temperature and oxygen stratification. Much of the shoreline is exposed to vigorous wave action, and submerged plants are restricted to protected bays. The bottom is rubble or sand in shallow areas grading into mud at depths >4 m (Mills et al. 1978). The general lack of vertical stratification and relatively small littoral zone implies that the lake could be approximated in model simulations by a single, well-mixed volume.

Data sources

We configured the IBM based on studies of the population

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Table 3. Predator–prey interactions defined in the walleye – yellow perch IBM by nonzero vulnerability factors.

	Yellow perch			Walleye			
Prey/predator	Larval	YOY, yearling	Adult	Larval	YOY	Yearling	Adult
Zooplankton	+	+	+	+	+[b]		
Benthos		+[a]	+	+	+	+	+
Forage fish					+	+	+
Larval yellow perch				+	+	+	
Larval walleye				+	+	+	
YOY yellow perch			+		+	+	+
YOY walleye					+	+	+
Yearling yellow perch						+	+
Yearling walleye						+	+

Note: YOY are juvenile YOY exclusive of larvae (i.e., >20 mm in length).
[a]Yellow perch >30 mm.
[b]Walleye <100 mm.

dynamics and trophic interactions of Oneida Lake walleye and yellow perch conducted from 1958 to 1991, before zebra mussel invasion (see Forney 1980 for review; Mills and Forney 1988). In most years, yellow perch were the dominant prey of walleye, and white perch and young walleye were of secondary importance as walleye forage (Forney 1974; Prout et al. 1990). Major shifts in the biomass of available walleye forage and irruptions by forage species (white perch and gizzard shad) occurred in Oneida Lake during the pre-zebra mussel period of record (1958–1991), which impacted the population dynamics of yellow perch and walleye (Table 1; Fig. 1). The "mayfly" period (1958–1969) was characterized by high densities of mayflies and strong, stable year-classes of walleye and yellow perch. The "baseline" period (1970–1978) was characterized by an absence of mayflies, generally low abundances of white perch, higher rates of walleye cannibalism, and lower and more variable recruitments of yellow perch and walleye. The "forage fish" period (1979–1991) was characterized by strong year-classes of YOY white perch or gizzard shad, higher recruitments of walleye, and lower recruitments of yellow perch. Similar adult abundances were observed for all three periods, with year-to-year variability lower during the mayfly and forage fish periods compared with the baseline period.

Differences in walleye and yellow perch mean abundances and survival rates between pre-zebra mussel periods (Mayfly, Baseline, Forage Fish) and the post-zebra mussel period were compared using ANOVA or a nonparametric Wilcoxon rank sums test. Assumptions of normality and homoscedasticity were tested before comparison of means. Differences in mean abundances or rates were considered significant at the $\alpha = 0.05$ level.

Model description

The model simulates the dynamics of yellow perch and walleye populations in Oneida Lake and is described in detail in Rose et al. (1999). The model begins with spawning of individual females of each species and simulates growth and survival of each female's progeny as they develop through successive life stages (egg, yolk-sac larva, feeding larva, YOY juvenile, yearling, and adult (age 2 and older)). Yellow perch are followed until age 10 and walleye until age 12. Predator–prey relationships represented in the model are described in Table 3.

The model represents daily dynamics in a single, well-mixed compartment 260 × 260 × 6.8 m deep scaled to represent Oneida Lake. The environment in the box consists of daily water temperature and the daily dynamics of a variety of prey types. Simulations were run for 50 years to simulate zebra mussel effects. Each year is 365 days long beginning on January 1.

Spawning

Adults are evaluated for spawning on day 100 (April 10) of each year. A female adult is mature if a generated random number is less the fraction mature for her length. Fraction mature and fecundity increase with female size for both species. Each female is assigned a temperature of spawning from species-specific probability distributions; a female spawns on the first day that simulated temperature exceeds the temperature of spawning.

Eggs and yolk-sac larvae

Each female's spawn of eggs is followed as a cohort. Day of hatching and day of first feeding are temperature dependent and determined by accumulating daily fractional development rates until the cumulative exceeds 1. Numbers of eggs or yolk-sac larvae in each cohort are reduced daily by specified daily mortality rates.

Feeding life stages

Initial conditions, development, and age: All first-feeding larvae are assigned identical initial lengths: 6.6 mm for yellow perch and 9 mm for walleye. Development from larvae to YOY juveniles (metamorphosis) occurs at 20 mm for both species. On April 10 each year, surviving individuals of each year-class are promoted to the next age.

Growth and feeding: Daily growth in weight is represented by the standard bioenergetics equation (Brafield 1985). We use parameter values from previously published bioenergetics models (see Rose et al. 1999). Maximum consumption and metabolism depend on an individual's weight and water temperature. Egestion, excretion, and specific dynamic action are computed as a fraction of realized consumption. An individual's weight is updated based on growth, and then, its new length is determined using length–weight relationships.

Realized consumption by individuals is based on a type II functional response relationship and is dependent on prey densities, vulnerability of each prey type to the individual predator, and half-saturation constants (K) governing the rate of predator saturation. Vulnerability-adjusted prey densities are obtained by either multiplying densities by a factor <1 (for zooplankton and benthos) or determining the vulnerability of each individual fish prey item (forage fish, yellow perch, and walleye) based on its length relative to the predator length. Values of K are specified for size intervals of the predator and each prey type and were determined by calibration (Rose et al. 1999).

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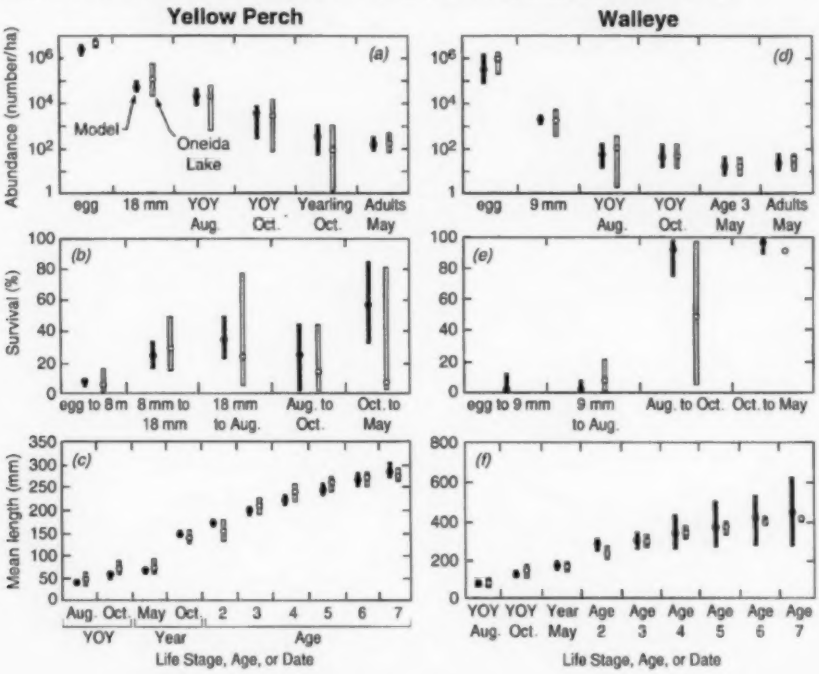
Fig. 3. Observed and simulated mean abundances, percent survival, and lengths by life stage, age, or date for (*a–c*) yellow perch and (*d–f*) walleye in Oneida Lake (Rose et al. 1999). Predictions of the IBM are shown on the left and observed data are shown on the right for each variable. Bars indicate ranges of mean values, and circles indicate averages of mean values.



A total of seven prey types are represented that encompass the dominant prey eaten by yellow perch and walleye from first feeding through the adult stage. Zooplankton are represented by the sum of small and medium *Daphnia*, benthos by chironomids, and forage fish by YOY white perch and a mix of smaller-sized species. The remaining four prey types, YOY and yearling life stages of yellow perch and walleye, are simulated as individuals in the model.

Each day, the densities of zooplankton, benthos, and forage fish are updated based on numbers consumed and a specified production rate using a modified logistic population growth function. Production rates of zooplankton, benthos, and forage fish are based on unpublished data for Oneida Lake and a recent review of benthic production and turnover rate (Berg and Hellanthal 1991). Equilibrium densities are the maximum densities of each prey type allowed in model simulations (i.e., carrying capacity in the logistic equation). Equilibrium densities of zooplankton are assumed to be time dependent based on the sum of small and medium *Daphnia* (<1.4 mm) measured weekly in Oneida Lake. Equilibrium densities of benthos and forage fish are assumed to be constants.

Mortality: Larval mortality is specified at fixed rates for each species. Mortalities of juvenile and yearling yellow perch and walleye are computed from the consumption rates of walleye and yellow perch. Adult (age 2 and older) mortality of yellow perch and natural mortality of walleye are assumed to be constant. Walleye fishing mortality decreases with increasing number of YOY yellow perch, walleye, and forage fish because walleye are less apt to be caught when their natural forage is available (Forney 1980; Lloyd 1996; Rose et al. 1999).

Model calibration and corroboration

We calibrated and corroborated the IBM using percid abundances, survival rates, growth rates, and diets from the Oneida Lake database. Model predictions of yellow perch and walleye abundances, mean lengths, and survival rates were subjectively judged to be similar to observed values (Fig. 3) (see Rose et al. 1999 for additional comparisons).

Baseline conditions

A 50-year simulation under baseline conditions without walleye stocking provided the basis for comparison of the effects of zebra mussels. In the model, no walleye were stocked (unless otherwise stated) to emphasize walleye – yellow perch interactions. Walleye stocking acts to partially uncouple walleye from yellow perch, as a constant number of walleye fry are added every year regardless of the state of walleye or yellow perch populations (Rose et al. 1999).

Design of zebra mussel effects simulations

ES effects

We performed three 50-year simulations to assess ES effects under baseline conditions. We imposed the ES effect and the zooplankton and benthos components separately. Under the ES hypothesis, we decreased zooplankton production rate and density by 20% and increased benthos production rate and density by 20%. Imposing each component separately allows determination of the relative importance of each component to the ES effect and provides information relevant to other ecosystems where changes in zooplankton and benthos may vary in importance. The 20% changes that we used seemed reasonable based on the limited data available and the high uncertainty associated with anticipated zebra mussel effects.

Robustness

We performed four additional 50-year simulations to establish whether predicted effects of zebra mussels were robust compared

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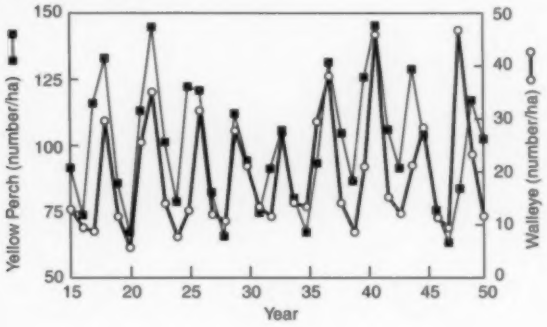
Fig. 4. Model-simulated abundances of adult walleye and yellow perch in Oneida Lake under baseline conditions (low forage fish densities, no mayflies).



with baseline conditions. All simulations used the ES changes to zooplankton and benthos. One simulation tested model sensitivity to altering the magnitude of the ES. Zooplankton density and production rate were reduced by multiplying by 0.7 (rather than 0.8), and benthos density and production rate were increased by multiplying by 1.5 (rather than 1.2). The remaining three simulations repeated the standard ES effect described above (0.8× for zooplankton, 1.2× for benthos) under conditions of either walleye stocking, increased densities of mayflies, or increased forage fish biomass. Walleye are stocked annually in Oneida Lake and are commonly stocked in other lakes. Mayflies were historically an important benthic prey in Oneida Lake and elsewhere and are beginning to repopulate western Lake Erie and Saginaw Bay. Forage fish densities could increase substantially again if the increase in aquatic macrophytes provided additional cover and protection from predators. High mayfly density conditions were simulated by setting benthos equilibrium densities to three times baseline, by not allowing walleye to consume YOY and yearling yellow perch and walleye, and by setting fishing mortality of walleye to zero. These changes were imposed between June 5 and July 19 each year corresponding to the period of peak mayfly emergence. Forage fish conditions were simulated by increasing equilibrium forage fish densities threefold. ES effects were then imposed on these altered mayfly and forage fish conditions.

Prediction variables

For continuity with model corroboration and other results presented in Rose et al. (1999), we report annual values of selected prediction variables corresponding to important life stages and time periods of walleye and yellow perch in Oneida Lake and elsewhere. October 15 marks the end of the growing season. The period of major walleye predation on YOY yellow perch occurs for early juveniles from the date that they average 18 mm (about June 11) to August 1 and for late juveniles from August 1 to October 15. We define recruitment as yearling abundance in October.

We averaged prediction variables for all simulations for years 15–50 and compared the averaged values from the ES simulation with those from the baseline simulation. We also predicted diets of walleye and yellow perch under the ES and baseline simulations. Diet composition was computed by individual wet weight (grams) for YOY, yearling, and adult (age 5 for walleye and age 4 for yellow perch) life stages separately.

For the remaining component and robustness simulations, we present results on six variables that characterized the major effects of zebra mussels predicted for the ES simulation. The selected variables were number of years of high recruitment, larval (9 mm for walleye and 18 mm for yellow perch) to recruit survival, recruit and adult abundance, adult mean length, and spawner abundance. For each of the four simulations, we plot the relative percent change from the baseline value of each of the six variables to facilitate comparison among different variables and across simulations.

Results

Baseline simulations

Abundances of walleye and yellow perch adults under baseline conditions without stocking exhibited coupled predator–prey dynamics (Fig. 4). An important feature of the baseline simulation is the number of years (9 out of 36) of high walleye recruitment (>55/ha) (Fig. 5*a*). Walleye recruitment was primarily a function of egg production (Fig. 5*c*). Average egg production for the 9 years of high recruitment was 708 986/ha compared with 194 308/ha for the remaining 29 years. High egg production coincided with years of longer adults, resulting in younger mean age at maturity and increased numbers of spawners. For example, in the 9 years of high recruitment, which coincided with years of high egg production, the fraction mature of age-5 walleye was 85%, age-5 walleye averaged 430 mm total length, and spawner density averaged 14.3 spawners/ha compared with the remaining 29 years when 7% of age-5 walleye were mature, age-5 walleye averaged 335 mm, and average spawner density was 4.1 spawners/ha. Exceptionally high adult abundance (Fig. 5*b*), larval survival (Fig. 5*d*), or 9 mm to recruit stage survival (Fig. 5*e*) was not necessary for high walleye recruitment.

Baseline simulations of yellow perch dynamics indicated that yellow perch recruitment was inversely correlated with adult walleye density. High yellow perch recruitment years, defined as >300 recruits/ha, occurred in 7 of 36 simulation years when adult walleye densities were relatively low and predation pressure on YOY yellow perch was reduced. In these years, adult walleye densities averaged 93/ha and mean survival rate of yellow perch during the 18 mm to recruit period was 2%. In years of lower yellow perch recruitment, mean density of adult walleyes was higher (143/ha), predation pressure on YOY was increased, and mean survival of yellow perch from 18 m to recruitment was fourfold lower (0.5%). No other yellow perch population variable, including egg production, larval density, and growth rate, differed significantly between years of high and low yellow perch recruitment. Average lengths of juvenile and recruit yellow perch were inversely correlated with recruitment success; YOY and recruited yellow perch were smaller during high-recruitment years (59 and 144 mm, respectively, versus 64 and 150 mm) owing to density-dependent competition for prey.

Effects of ES

Imposition of the ES changes resulted in reduced walleye recruitment and adult abundance because of fewer years of high walleye recruitment and lower larval growth, which led to lower larval survival (Table 4; Fig. 5). The ES simulation resulted in only 3 years of high walleye recruitment (>55/ha) compared with 9 years under baseline conditions. Average recruitment and adult walleye abundance under the ES simulation were nearly 27 and 32% lower, respectively, than corresponding baseline values (Figs. 5*a* and 5*b*). Average egg production under the ES simulation was 18% lower than baseline, and the ES simulation also had fewer years of high

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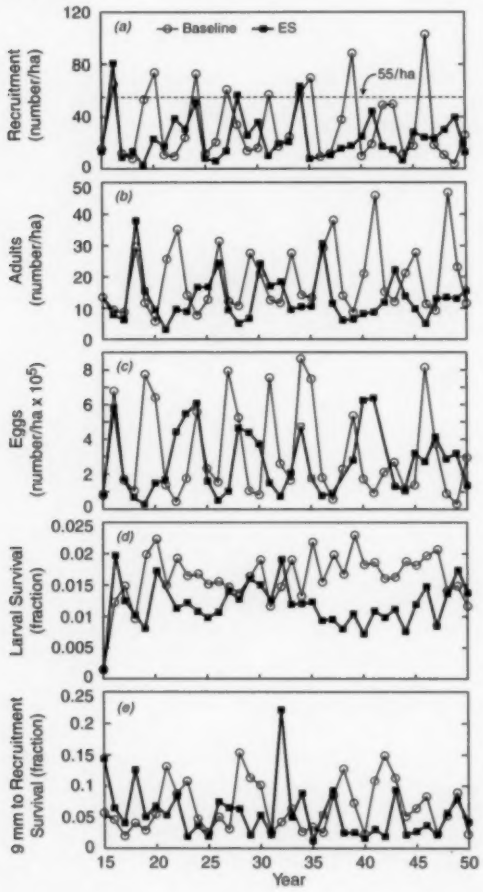
Fig. 5. Model predictions of walleye (*a*) recruitment, (*b*) adult abundance, (*c*) egg abundance, (*d*) larval survival rate, and (*e*) survival from 9 mm total length to recruitment under baseline and ES conditions.



egg production than under baseline conditions (Fig. 5*c*). The loss of high-recruitment years was due to negative effects of lowered zooplankton densities on larval walleye growth and survival. The lowered zooplankton densities lowered larval growth rates from 0.68 mm/day under baseline to 0.64 mm/day and decreased survival rates during the larval stage (Fig. 5*d*) from 0.0166 under baseline to 0.0121. Lower larval survival led to lower survival from 9 mm to recruitment (Fig. 5*e*).

Walleye exhibited compensatory responses in juvenile and adult growth rate and adult reproduction that were insufficient to offset the reduction in larval survival. Average lengths of age-5 walleye increased in the ES simulation due to increased consumption by yearlings and adults. Mean walleye lengths under ES conditions were 16% longer for recruits and 21% longer for age-5 adults; growth rate of age-5 walleye increased by 31% (Table 4). Increased growth of adults lowered the age at maturation (58% of age-5 females were mature under ES conditions versus 33% under baseline). The younger age at maturation and increased fecundity offset some, but not all, of the reduction in adult abundances and resulted in a 22% reduction in spawner density (Fig. 6*a*). The lower mean abundance of adult walleye under the ES treatment reduced yield in numbers by 29% compared with baseline values. Yield in biomass declined by only 9% because of increased mean length at age under ES (Table 4).

ES effects on yellow perch also lowered growth and increased mortality for yellow perch larvae but reduced walleye predation, resulting in yellow perch recruit and adult abundances that were similar to baseline values (Fig. 6*b*; Table 5). Larval yellow perch survival declined by 52% compared with baseline (from 28.7 to 13.9%), due to the negative impact of lowered zooplankton on larval growth. As a result, average 18-mm larval abundances were nearly half those under baseline conditions. Decreased survival of yellow perch larvae was approximately offset by increased survival of juvenile yellow perch from August to October, due to lowered walleye adult abundances that reduced walleye predation on yellow perch. The net effect was an 85% increase in survival from 18 mm to recruitment (Fig. 6*b*), which resulted in abundances of YOY in October, recruits, and adults that were similar to or greater than baseline values (Table 5). Mean growth rate of YOY yellow perch was lower through August but increased thereafter, resulting in longer recruits and similar-sized adults (Table 5). Similar sizes and abundances of adults under ES lowered egg production by only 7% from baseline levels.

The ES simulation caused shifts in yellow perch diets (Fig. 7) and walleye diets (Fig. 8). Under ES changes, percentages of zooplankton in the diet decreased, and benthos increased, for all stages of yellow perch and for YOY and yearling walleye. For yearling yellow perch, percent composition by weight of benthos increased from 62 to 74%, while percent composition of zooplankton decreased from 38 to 26% (Fig. 7). Yearling yellow perch also increased total weight of prey consumed by 21% over baseline, while adults decreased weight consumed by 14%. The percentage of benthos in yearling walleye diets increased (from 56 to 63%), and the percentage of YOY yellow perch decreased (from 22 to 16%) (Fig. 8). Adult walleye diets shifted from roughly equal percentages of YOY and yearling yellow perch under baseline to a dominant proportion of yearling yellow perch. Total weight of prey consumed increased by about 10 and 50% for YOY and yearling walleye but was similar for adult walleye under the ES simulation.

Effects of ES components: zooplankton and benthos

Prediction of the total ES effect required both components of reduced zooplankton and increased benthos (Tables 4 and 5; Fig. 9). Increased benthos alone affected walleye and yellow perch dynamics, but predicted responses did not resemble the combined ES response. Walleye variables that declined under ES changes declined less under benthos alone, and several actually increased. Compared with declines in walleye abundances and survival predicted under ES, the number of high-recruitment years, 9 mm to recruit survival, and abundances of recruits, adults, and spawners either decreased less or increased under the increased benthos alone simulation (Fig. 9*a*; Table 4). Mean adult lengths and growth rates of age-5 walleye were similar to baseline values. Increased benthos plays an important role in

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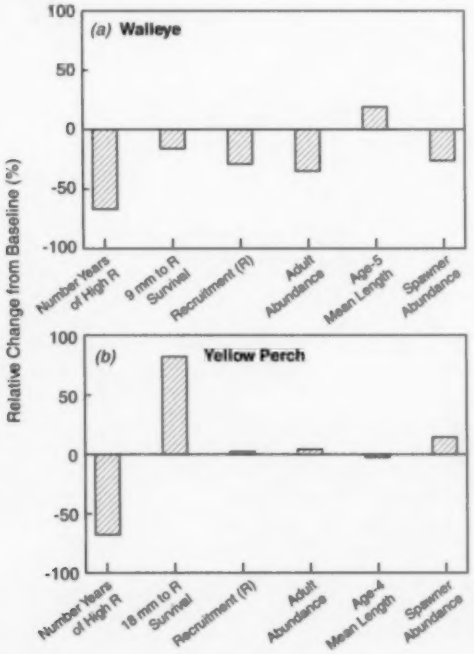
Table 4. IBM simulation results of baseline, ES, and ES components for walleye.

Variable	Stage	Simulation			
		Baseline	ES	0.8 × Z alone	1.2 × B alone
No. of high-recruitment years	Recruits	9	3	4	7
Abundance (no./ha)	Eggs ($\times 10^6$)	0.331	0.271	0.218	0.364
	9-mm larvae	736	618	469	747
	Fall YOY	39.8	26.7	26.5	50.7
	Recruits	33.2	24.1	22.7	34.7
	Adults	19.9	13.5	12.5	20.5
	Spawners	6.7	5.2	3.7	7.7
Total length (mm)	Oct. YOY	129	139	131	138
	Recruits	244	284	259	272
	Age 5	365	441	449	396
Growth (mm/year)	Age 5	35.3	46.1	67.6	32.4
Yield (kg/ha)	Adults	8.5	7.8	7.2	8.9
% survival	9 mm to recruit	6.56	5.56	6.13	6.56
% maturity	Age 5	33	58	57	46

Note: Variables include number of years that recruitment exceeded 55/ha, abundance, total length, growth rate, yield, percent survival from 9 mm to recruit, and percent mature of age-5 adults. Values represent averages of 50-year simulations. The ES simulation combines both decreased zooplankton density and production rate (0.8 × baseline zooplankton levels (0.8 × Z)) and increased benthos and production rate (1.2 × baseline benthos levels (1.2 × B)).

Fig. 6. Model predictions of the ES effect on (*a*) walleye and (*b*) yellow perch abundances (no./ha), survival rates (fraction), and mean total lengths (mm). Predication variables evaluated are the number of years of high recruitment, survival from larval stage (9 or 18 mm) to recruitment, recruitment, adult abundance, adult mean length, and spawner abundance. Effects are expressed as relative percent difference between the mean value under ES (Y_{ES}) and the mean value under baseline conditions (Y_B) ($100 \times (Y_{ES} - Y_B)/Y_B$).



providing an alternative prey for YOY and yearling walleye, enabling recruits to grow longer than under baseline conditions, despite reduced zooplankton.

Slightly higher than baseline abundances and lengths of walleye recruits and adults under the increased benthos alone simulation resulted in increased predation pressure on yellow perch. Increased predation by walleye decreased the number of years of high yellow perch recruitment, greatly lowered survival in the 18 mm to recruit stage, and lowered recruitment and adult yellow perch abundances compared with the ES simulation (Fig. 9*b*; Table 5). Spawner abundances remained 16% above baseline values because of increased mean lengths of adults.

The component of reduced zooplankton alone came closer to mimicking the same total response for walleye as predicted by the ES simulation, but predicted responses still differed for yellow perch. While the relative magnitudes of predicted changes in walleye were not identical to those predicted for ES combined, the direction of changes was fairly consistent. As with ES changes, reduced zooplankton alone led to fewer years of high walleye recruitment compared with baseline, reduced 9 mm to recruitment survival, lower recruit and adult abundances, and longer adult mean lengths (Fig. 9*a*). Walleye spawner density under reduced zooplankton alone conditions decreased by 45% below baseline compared with a 22% reduction below baseline under ES conditions. For yellow perch, reduced zooplankton alone produced a smaller decrease in the number of years of high yellow perch recruitment compared with the decrease predicted under the ES simulation (Fig. 9*b*). Predicted survival during the 18 mm to recruit stage also was much higher under the reduced zooplankton simulation than under the ES simulation. The much higher survival from 18 mm to recruit under the reduced zooplankton alone simulation led to higher abundances of recruits and adults than under ES combined and a minor decrease in adult yellow perch mean lengths. The decrease in adult mean lengths lowered the spawner density compared with the ES simulation.

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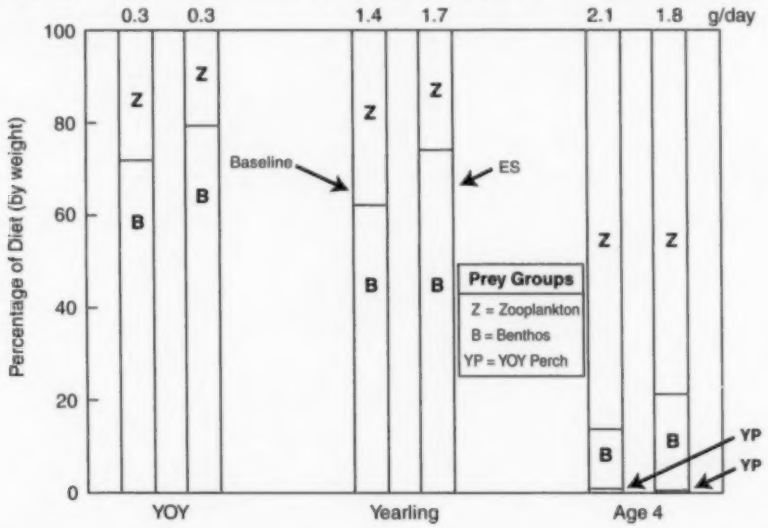
Table 5. IBM simulation results of baseline, ES, and ES components for yellow perch.

Variable	Stage	Simulation			
		Baseline	ES	0.8 × Z alone	1.2 × B alone
No. of high-recruitment years	Recruits	7	2	4	1
Abundance (no./ha)	Eggs ($\times 10^6$)	1.11	1.03	0.64	1.43
	18-mm larvae	31 370	16 933	11 263	38 655
	Fall YOY	3 360	3 128	2 932	2 908
	Recruits	180	182	294	114
	Adults	95	102	134	76
	Spawners	35.4	41.5	29.4	41.2
Total length (mm)	Oct. YOY	63	64	60	67
	Recruits	149	157	146	162
	Age 4	216	211	194	233
Growth (mm/year)	Age 4	21.3	13.4	12.9	21.6
Yield (kg/ha)	Adults	5.6	5.5	6.5	5.1
% survival	18 mm to recruit	0.61	1.13	2.89	0.31
% maturity	Age 4	43	49	16	77

Note: Variables include number of years that recruitment exceeded 300/ha, abundance, total length, growth rate, yield, percent survival from 18 mm to recruit, and percent mature of age-4 adults. Values represent averages of 50-year simulations. The ES simulation combines both decreased zooplankton density and production rate (0.8 × baseline zooplankton levels (0.8 × Z)) and increased benthos and production rate (1.2 × baseline benthos levels (1.2 × B)).

Fig. 7. Model-predicted diets of YOY, yearling, and age-4 yellow perch under baseline (shown on left) and ES conditions (shown on right). Diet composition by prey category is expressed as the percentage of body weight consumed per individual per day.



Robustness

Predicted responses by walleye and yellow perch variables were relatively robust to the magnitude of changes imposed on zooplankton and benthos, stocking, and increased forage fish conditions. Reducing zooplankton by 30% (rather than 20%), increasing benthos by 50% (rather than 20%), and imposing ES effects under stocking and increased forage fish generated responses similar to those of the ES effect under baseline conditions (Fig. 10). The major differences were that spawner abundance of walleye increased and recruit and adult abundances of yellow perch decreased under the larger ES effect and increased forage fish simulations.

Increasing mayfly densities offset most of the ES effects predicted for walleye and yellow perch by buffering predation and increasing survival during the juvenile stage for both species (Fig. 10). The number of years of high walleye recruitment, survival from 9 mm to recruitment, and abundances of recruits and adults were much higher under ES imposed on mayfly conditions than under ES imposed on baseline conditions (Fig. 10*a*). For yellow perch, the number of high-recruitment years and postlarval survival from 18 mm to recruit were much higher than predicted under ES, which greatly increased recruitment and adult abundances (Fig. 10*b*).

Discussion

Model predictions

Model simulations indicated that impacts of an ES from zebra mussel invasion on Oneida Lake walleye and yellow perch populations may be substantial but not catastrophic.

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Fig. 8. Model-predicted diets of YOY, yearling, and age-5 walleye under baseline (shown on left) and ES conditions (shown on right). Diet composition by prey category is expressed as the percentage of body weight consumed per individual per day.

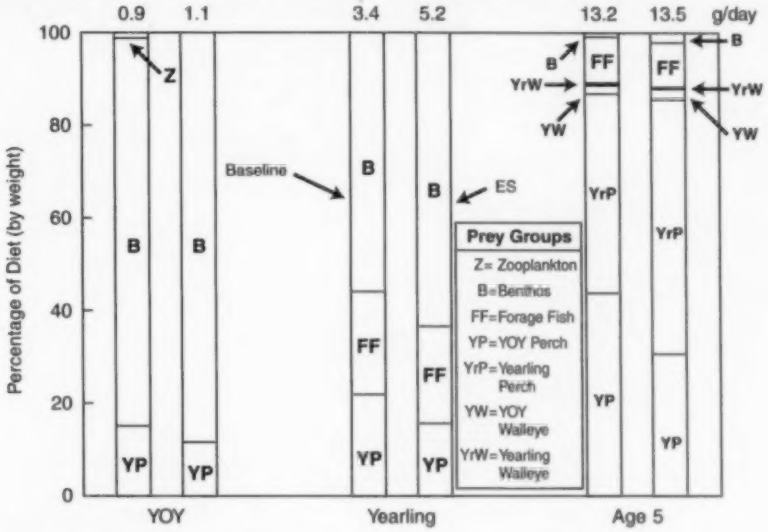
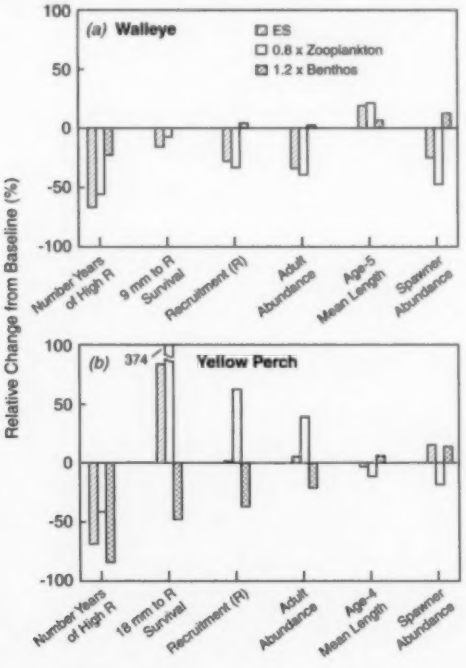


Fig. 9. Model predictions of the effects of ES and the zooplankton and benthos components for (*a*) walleye and (*b*) yellow perch. Prediction variables were the number of years of high recruitment, survival from larval stage (9 or 18 mm) to recruitment, recruitment, adult abundance, adult mean length, and spawner abundance. Effects are expressed as relative percent difference between the mean value under ES (Y_{ES}) and the mean value under baseline conditions (Y_B) ($100 \times (Y_{ES} - Y_B)/Y_B$).



The major predicted impacts were fewer years of high walleye recruitment due to increased larval mortality, which resulted in declines in recruitment and adult abundance of about 30% and reductions in yield (kilograms per hectare) of about 9%. Corresponding effects of an ES on yellow perch ultimately were dominated by indirect effects via changes in walleye predation pressure. Although predicted effects of an ES caused a population bottleneck in the larval stage for yellow perch, higher survival of postlarval stages (due to reduced walleye predation) led to slight increases in abundances of yellow perch recruits and adults over baseline values. Predicted effects of an ES on yellow perch and walleye were subtle and underscore the value of a modeling approach for assessing population effects, even in the apparently simple predator–prey community of Oneida Lake.

Our prediction of reduced walleye recruit and adult abundances and increased yellow perch recruit and adult abundances was common to many of the simulations performed. Robustness analysis showed that walleye stocking and increased densities of forage fish would not significantly change predicted effects of an ES. Only in simulations in which benthos densities alone were increased or mayfly densities were increased, both acting to buffer predation on YOY fish, did walleye abundances not decline or yellow perch abundances not increase.

Both components of decreased zooplankton and increased benthos were required to generate the total response by walleye and yellow perch to ES changes. The simulated zooplankton alone effect was generally larger than the benthos alone effect, but this may have been due to the relative magnitudes of the changes that we imposed. Both levels of changes in zooplankton and benthos yielded consistent response patterns. While our results are undoubtedly sensitive to the relative changes imposed, the changes in zooplankton and benthos that we did use are realistic based on empirical information from many lake ecosystems. Studies have documented 50–75% reductions in microzooplankton biomass

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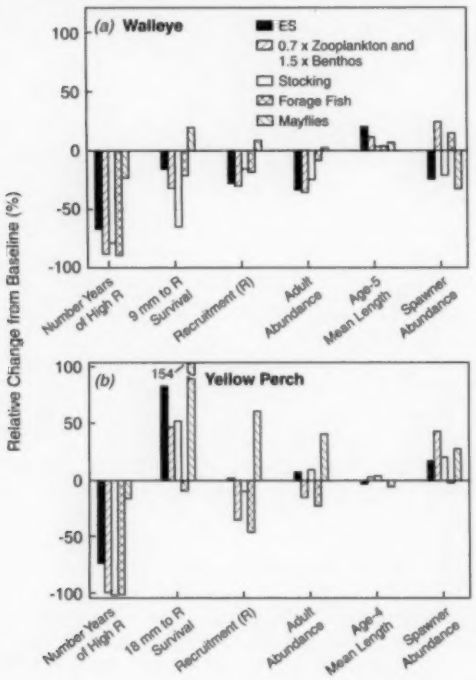
Fig. 10. Model predictions of the effects of ES for the robustness simulations for (*a*) walleye and (*b*) yellow perch. Robustness simulations were ES simulated as larger changes in zooplankton densities and turnover rates (0.7× rather than 0.8×) and benthos densities and turnover rates (1.5× rather than 1.2×), walleye stocking, increased forage fish density, and increased densities of mayflies. Prediction variables were the number of years of high recruitment, survival from larval stage (9 or 18 mm) to recruitment, recruitment, adult abundance, adult mean length, and spawner abundance. Effects are expressed as relative percent difference between the mean value under ES (Y_{ES}) and the mean value under baseline conditions (Y_B) ($100 \times (Y_{ES} - Y_B)/Y_B$).



(Bridgeman et al. 1995) and 100% increases in some benthic taxa for Saginaw Bay (Griffiths 1993; Nalepa and Fahnenstiel 1995) and Lake Erie (Stewart and Haynes 1994). The simulated changes in macrozooplankton density may not be realistic for Oneida Lake (Idrisi 1997) and some other lakes (Wu and Culver 1991), where phytoplankton and *Daphnia* productions have not changed since zebra mussel invaded, but are realistic for some eastern European lakes that have experienced declines in phytoplankton and macrozooplankton biomass as a result of zebra mussel invasion (Karateyev et al. 1997). The relative importance of the different components likely will vary among communities and systems.

Our model simulations support the hypothesis that the ES would create a population bottleneck for yellow perch (Persson and Greenberg 1990). Model-simulated reductions in zooplankton density did reduce growth in the planktonic larval and juvenile stages but resulted in a bottleneck for yellow perch in the larval stage. This scenario may be realistic in some Great Lakes ecosystems where prey organisms (copepod nauplii, rotifers) for first-feeding yellow perch have decreased by 50–75% since the arrival of the zebra mussel (Nalepa and Fahnenstiel 1995) but is unlikely to have occurred in Oneida Lake where macrozooplankton density has not changed and larval survival rates have increased. The short-term declines of larger zooplankton taxa have been documented in Saginaw Bay (Bridgeman et al. 1995) and in eastern Europe but have not been documented in other Great Lakes watersheds. If zebra mussel effects on the zooplankton community are confined to taxa preferred by first-feeding larvae, then population-level effects may be less than we predicted.

A corollary hypothesis of the population bottleneck was that growth, survival, and abundances of yearling and adult yellow perch would increase because of the increased density of benthos. Recent laboratory and mesocosm studies of zebra mussel effects give conflicting results about the effects of zebra mussels on fish growth. Thayer et al. (1997) and Richardson and Bartsch (1997) demonstrated increased growth of yearling yellow perch and juvenile bluegill (*Lepomis macrochirus*), respectively, in the presence of zebra mussels. In contrast, growth of fathead minnow (*Pimephalus promelas*) juveniles (Jennings 1996) and prey capture success of yellow perch were reduced in the presence of zebra mussels (Mayer 1998). Our model simulations predicted increases in growth rate and size of fall YOY and yearling yellow perch, but the increased size had less effect on their survival and recruitment than did the reduction in walleye predation caused by the ES effects on survival and growth of walleye larvae.

Model–data comparisons

Despite the availability of the long-term database at Oneida Lake, it is difficult to state unequivocally that an ES effect from zebra mussels has influenced percid dynamics because of lagged time effects from species irruptions that occurred earlier and the short length of the post-zebra mussel invasion database. A simple comparison of the pre- and post-zebra mussel invasion response in walleye and yellow perch abundances, growth, and survival rates provides ambiguous evidence for the effects of the ES (Figs. 1 and 2; Table 1). Whether survival of pelagic YOY life stages of yellow perch (egg to 18 mm, 18 mm to August) has decreased and survival of YOY demersal stages (August–October; October–October at age 1) has increased depends on the period selected for preinvasion and the life stages examined. Indeed, selection of the most recent period (1986–1991) as the preinvasion period suggests that zebra mussels have had a positive effect on survival of the pelagic YOY life stages and a negative effect on YOY demersal life stages. Overwinter survival (October–October) of YOY yellow perch has increased only slightly since the late 1980s but is much higher than mean survival rates observed in earlier periods.

Field data support model predictions of ES effects causing increased YOY yellow perch growth. Mean length of YOY yellow perch in October increased from 78 ± 6 mm (3–4 g) in 1986–1991 (before zebra mussel invaded) to 82 ± 6 mm (6–8 g) after 1992. Densities of Cladocera have not changed since zebra mussel arrived, but their availability to yellow perch may have increased due to the increased water clarity in Oneida Lake and a 67% reduction in planktivore biomass compared with the 1970s and 1980s. Densities of most shallow-

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water benthic taxa in Oneida Lake have increased 10-fold since zebra mussel invaded. YOY diets have not changed, but adult diets show greater incidence of zooplankton and benthos (Mitchell et al. 1996; Mayer 1998). The increased size of YOY yellow perch in fall has greatly reduced overwinter (October–October) mortality rates and increased recruitment in recent years (Table 1) but has not resulted in increased adult populations. A possible explanation for why adult yellow perch abundances have not rebounded is the irruption of another piscivore, the double-crested cormorant (*Phalocrocorax auritus*). Cormorants target yellow perch in the yearling and young adult size range and are now estimated to consume nearly 15% of the adult yellow perch stock (C. Adams, Cornell University, Ithaca, N.Y., unpublished data).

Data on walleye qualitatively support model predictions of declines in walleye abundances but not predicted increases in walleye growth. Walleye dynamics since zebra mussels invaded are consistent with the hypothesis that lack of available forage will lower first-year growth and decrease overwinter survival and recruitment (Forney 1974). The fast growth and low abundance of YOY yellow perch have restricted their availability to YOY walleye to only a few weeks and lowered YOY walleye predation on yellow perch. As a consequence, walleye growth rates have declined and cannibalism rates have increased. In contrast with model predictions of increased walleye growth, fall length of YOY walleye decreased from a mean (±2 SE) of 160 ± 16 mm before zebra mussel invasion to a mean of 149 ± 7 mm afterward. Thus, model predictions of a reduction in walleye recruitment and adult abundance arise from different reasons than observed in Oneida Lake. The model predicted that walleye densities would decline because of reduced larval growth and survival, not from reduced availability of fast-growing YOY yellow perch and increased cannibalism. The model predictions of reduced larval walleye growth are unlikely to have occurred in Oneida Lake, where preferred Cladocera prey densities (Mayer and Wahl 1997) have not declined. Increased growth of model juvenile walleyes, which resulted from increased benthos densities alone, also is unlikely because YOY and yearling walleye do not grow well on benthos (Forney 1966).

Concluding remarks

Our model simulations demonstrate the hypothesized impacts of an ES on percid populations resulting from zebra mussel invasion. Although many of the predicted effects from the combined ES simulation are qualitatively being realized in Oneida Lake, the data also suggest some discrepancies. Rather than the predicted minor change in yellow perch recruitment, the immediate impact of zebra mussel invasion in Oneida Lake may have been to increase growth of YOY yellow perch, thereby reducing their availability to YOY walleye and resulting in an increase in yellow perch recruitment. Our model simulations indicate that the ES would lower walleye recruitment by reducing larval growth and survival rates, not by reducing vulnerability of YOY yellow perch to walleye predation. The model simulations do indicate that, regardless of the causes, increased mortality in early life stages can have major effects on percid populations.

Other zebra mussel impacts not explored in our analysis also could cause changes in the mortality rates of early life stages, and their inclusion, where appropriate, could improve model forecasts. Zebra mussels have substantially increased water clarity in Oneida Lake, which has promoted macrophyte growth (Mayer 1998). In the Thames River tributary to Lake St. Clair, the decline in abundance of walleye larvae is coincident with zebra mussel invasion, possibly as a consequence of increased predation by planktivores utilizing increased macrophytes (R. Griffiths, Ontario Ministry of Natural Resources, personal communication). Macrophytes may increase YOY yellow perch survival by providing additional refuge from walleye predation. Increased water clarity also may increase YOY yellow perch feeding success by lowering reactive distances to zooplankton prey. Other potential impacts of zebra mussels not simulated in our analysis were the effects of zebra mussels as substrate for walleye egg deposition (Fitzsimons et al. 1995) and the influence of blue-green blooms (Idrisi 1997). Finally, aspects of the model, such as overdependence of walleye on benthos and the size dependence of overwinter mortality of yellow perch (see Rose et al. 1999), can be improved. More realistic representation of zebra mussel effects and improvements in the model would increase the accuracy and confidence in model forecasts of zebra mussel effects.

Zebra mussel impacts on percid populations may not be fully understood for some time. Recent surveys of zebra mussel colonization in western Lake Erie (D.W. Schloesser, USGS-BRD, Great Lakes Science Center, Ann Arbor, Mich., personal communication) and Oneida Lake (Mayer 1998) indicate that zebra mussels have now colonized soft substrates in addition to hard substrates. Further expansion by zebra mussels will increase filtration of edible phytoplankton and may impact macrozooplankton densities, which, to date, have not been affected in Great Lakes ecosystems. In some eastern European lakes, negative effects of zebra mussel filtration on phytoplankton and zooplankton biomass were not observed until 10 years postinvasion (Karateyev et al. 1997). The increased growth of zoobenthos and epiphytic algae, in combination with decreasing phosphorus levels, may serve to limit pelagic production or alter pathways of energy flow available to fish (Nalepa and Fahnenstiel 1995). Our modeling approach should help identify changes in percid population dynamics experiencing zebra mussel invasions. These changes can be used as early-warning signals of possible long-term consequences of zebra mussel invasions in lake ecosystems.

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Influence of large woody debris and a bankfull flood on movement of adult resident coastal cutthroat trout (*Oncorhynchus clarki*) during fall and winter

Bret C. Harvey, Rodney J. Nakamoto, and Jason L. White

Abstract: To improve understanding of the significance of large woody debris to stream fishes, we examined the influence of woody debris on fall and winter movement by adult coastal cutthroat trout (*Oncorhynchus clarki*) using radiotelemetry. Fish captured in stream pools containing large woody debris moved less than fish captured in pools lacking large woody debris or other cover. Fish from pools lacking cover commonly moved to habitats with large boulders or brush, particularly during the day. Movements by fish over 1-day periods were strongly influenced by large woody debris or other elements providing cover. Fish initially found in habitats lacking large woody debris, large boulders, or brush cover moved the most extensively, while fish initially found in pools with large woody debris moved the least. Fish did not move extensively in response to a bankfull flood, although some moved to habitat downstream of large woody debris in tributaries or secondary channels. Habitat downstream of woody debris in the main channel was not used during the flood, apparently because of extreme turbulence. Overall, these observations provide additional evidence for the value of habitat complexity to some stream fishes and support previous observations of minimal effects of flooding on adult fish.

Résumé : Afin d'améliorer notre connaissance de l'importance des gros débris ligneux pour les poissons des cours d'eau, nous avons examiné, par radiotélémétrie, l'influence des débris ligneux sur les déplacements d'automne et d'hiver de truites fardées côtières adultes. Les poissons capturés dans les fosses de cours d'eau où se trouvaient de gros débris ligneux se déplaçaient moins que ceux capturés dans les fosses exemptes de tels débris ou d'un autre couvert. Les poissons des fosses sans couvert se déplaçaient généralement vers des habitats où se trouvaient de grosses pierres ou des broussailles, cela surtout pendant le jour. Les mouvements des poissons au cours d'une même journée étaient fortement influencés par la présence de gros débris ligneux ou d'autres éléments offrant un couvert. Les poissons des habitats exempts de gros débris ligneux, de grosses pierres ou de broussailles sont ceux qui se déplaçaient le plus tandis que ceux des fosses à gros débris ligneux se déplaçaient le moins. Les poissons ne se sont pas déplacés de façon importante suite à une crue affectant toute la rive, bien que certains se soient déplacés vers des habitats se trouvant en aval de gros débris ligneux, dans des tributaires ou des chenaux secondaires. Les habitats en aval des débris ligneux du chenal principal n'étaient pas utilisés pendant les crues, sans doute à cause de la turbulence extrême. De façon générale, ces observations confirment l'importance de la complexité de l'habitat pour certains poissons de cours d'eau et complètent des observations antérieures du peu d'effets des crues sur les poissons adultes.

[Traduit par la Rédaction]

Introduction

Large woody debris in streams affects the habitat of fishes. Scour around large woody debris can create pools, increasing the density of fishes that prefer pool habitat (Fausch and Northcote 1992). By increasing the visual isolation of individuals, woody debris may allow higher densities of territorial fishes (Dolloff 1986). Woody debris may also provide habitat with relatively low risk of predation (e.g., Angermeier and Karr 1984) or refuge from high water velocities (McMahon and Hartman 1989). In coastal, temperate-zone streams, large woody debris probably has its largest impact on predation risk and its greatest significance as a physical refuge in winter, when cold temperatures reduce swimming performance (Webb 1978) and high discharge increases water velocities. Indeed, during the winter, coho salmon (*Oncorhynchus kisutch*) in Oregon streams are most abundant in habitats usually associated with large woody debris, whereas they are more evenly distributed among habitats in other seasons (Nickelson et al. 1992*a*). Retention of coho salmon after winter freshets was positively related to the volume of woody debris in sections of Carnation Creek, British Columbia (Tschaplinski and Hartman 1983).

Observations of fish movement in the vicinity of large woody debris may illuminate the mechanisms of its significance to fishes. For example, where large woody debris provides refuge from high water velocity during floods,

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aggregations of individuals behind woody debris (McMahon and Hartman 1989) or relatively little movement by individuals occupying habitats with woody debris might be expected. Movement rates of territorial fish during baseflows might be relatively low at sites with large woody debris if it provides superior habitat (Winker et al. 1995).

To our knowledge, no winter field observations addressing these possibilities are available. Our objective in this study was to quantify the influence of large woody debris on movement and use of habitat by adult coastal cutthroat trout (*Oncorhynchus clarki*) across a range of discharges in fall and winter. In fulfilling this objective, we also added to the small number of observations of movement by individual stream fish during floods (e.g., Todd and Rabeni 1989; Matheney and Rabeni 1995).

Study site and methods

Little Jones Creek is a third-order tributary of the Middle Fork Smith River in northwestern California (41°51′N, 123°49′W), draining about 2750 ha of steep forested terrain. About 30% of the watershed has been logged in the last 50 years. The study reach, from 1400 to 3500 m upstream of the confluence with the Middle Fork Smith River, has a nearly complete canopy of red alder (*Alnus rubra*). Stream gradient in the study reach averages 1.8%. About 45% of the pools in the study reach were formed by scour adjacent to large woody debris >30 cm in diameter; the rest were adjacent to bedrock outcrops. The stream averaged 8 m in width during common winter flows of about 1.5 $m^3 \cdot s^{-1}$. At the site where we measured discharge during this study, depth averaged about 35 cm and velocity about 45 $cm \cdot s^{-1}$ at this flow. During a bankfull flood on 16–17 January 1998, discharge rose to about 45 $m^3 \cdot s^{-1}$. At the site where we measured discharge, stream width reached about 11 m during the flood, while depth averaged about 1.2 m and water velocities reached >2.7 $m \cdot s^{-1}$. Water temperature ranged 5.5–11.0°C during this study (February–March 1997 and October 1997 – February 1998). Coastal cutthroat trout is the only fish species in the stream.

We collected fish for the study by electrofishing in two kinds of habitats: pools formed by scour adjacent to bedrock, which contained little or no cover for fish (classified as "simple" habitat), and sites with abundant large woody debris ("complex" habitat). We usually collected fish at night because previous sampling efforts in winter suggested higher capture efficiency at night compared with daytime. On each collecting date (24 February 1997, 15 October 1997, 12 November 1997, 11 December 1997, and 9 January 1998), we implanted 1.2-g radiotransmitters in 8–10 fish, four or five fish from each of the two kinds of habitats. We tagged a total of 24 fish from simple pools and 22 fish from complex pools (three individuals were not included in the analyses because we were unable to make adequate observations on them). Cutthroat trout that received transmitters ranged from 177 to 236 mm fork length and from 60 to 138 g. Fish from the two habitat types were similar in size: both groups averaged 199 mm fork length and 89 g.

Transmitters were surgically implanted into the body cavity using the technique described by Young (1995). Transmitters and coiled antennae formed single units sealed in epoxy. They remained viable for about 30 days, and we tracked individual fish for an average of 29 days. We observed each fish during at least four 24-h visits to the site. During each visit, we located fish at least twice during the day and twice at night, with observations separated by at least 4 h. Usually, we made an initial daytime observation followed by two nighttime observations and a second daytime observation.

We were unable to follow this protocol during the bankfull flood on 16–17 January 1998, when logistical difficulties prevented us from gathering data rapidly enough to make the normal complement of observations. During that event, we were able to locate all fish with functional transmitters at least three times within 24 h, when discharge exceeded 30 $m^3 \cdot s^{-1}$ and stage was within 12 cm of the maximum attained during the flood.

We located fish by triangulation with a receiver and Yagi antenna while walking the streambank and wading. In general, we located fish within 1 m^2, as indicated by our success in recapturing fish with viable transmitters by electrofishing. Locations were occasionally less precise in the vicinity of large bedrock outcrops. For each observation, we determined the longitudinal position within the reach by referring to flagging placed every 25 m and noted the type of habitat occupied.

We also collected water temperature and stream-flow data because of their potential significance to fish movement. A data logger positioned near the middle of the study reach recorded water temperature every 15 min during the study. In November 1997, we placed a staff gage in the stream and measured stage during subsequent visits to the study site. We measured discharge on 20 occasions to establish a stage–discharge relationship. To obtain information on discharge in Little Jones Creek when direct measurements were unavailable, we attempted to predict stage at Little Jones Creek using the stage of the mainstem Smith River at a U.S. Geological Survey gage 28 km downstream. We performed a series of regressions using the stage of the mainstem Smith River from 0 to 6 h after our observations of stage at Little Jones Creek and used the strongest relationship to predict stage in Little Jones Creek. For this analysis, we used a set of 55 observations of Little Jones Creek stage made on separate days.

We compared the site fidelity of fish captured from complex versus simple pools in several ways. First, we computed the proportion of observations where each fish was in the specific habitat where it was initially captured. We transformed these proportions (arcsin square root) and then compared fish from the two kinds of habitats with a t test. Second, we compared the distance between the point of capture and the point of last observation for fish from the two kinds of habitats, also using a t test. A $\log(x + 1)$ transformation of these distances homogenized the variances of the two treatments. Finally, we examined the subsequent use of different habitat types by fish captured in the two different types of pools. For this comparison, we recognized four habitat types: (*i*) "open" habitat with no large (>75 cm long axis) boulder or wood cover for fish, (*ii*) pool habitat with cover provided by large woody debris, (*iii*) riffle or run habitat with cover provided by brush or alder roots, and (*iv*) riffle or run habitat with cover provided by boulders (>75 cm long axis). Habitats classified as open often included areas with unembedded cobbles and small boulders that probably provided cover for fish (Meyer and Griffith 1997). The open habitat category also included both fast- and slow-water habitats.

We also compared the movement behavior of fish from different kinds of habitats by analyzing changes in their positions during the 1-day observation periods. We measured these changes in two ways. First, we quantified the length of stream over which individual fish were observed during each period. Second, because significant nighttime movements by fish sometimes resulted in little "net movement" from one day to the next, we calculated the change in position of individual fish on successive days. Because some fish rarely were observed in the habitat where they first were captured, and most fish used various types of habitat over the course of our observations, we included the habitat type occupied by fish when first located during the 1-day observation periods as a factor in the analyses. We used the four habitat types described above to classify these and employed an analysis of covariance design that accounted for repeated observations of individual fish (Table 1). River stage and water temperature were included as covariates in these analyses.

Because only one bankfull flood occurred during this study, we

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Table 1. Outline of the analysis of covariance used to analyze movement by radiotagged adult coastal cutthroat trout over 1-day observation periods in Little Jones Creek, California.

Source of variation	df	Error term
Capture site habitat	1	Fish within capture site habitat
Fish within capture site habitat	41	
Initial habitat (at beginning of 1-day observation periods)	3	Fish within capture site habitat × initial habitat
Initial habitat × capture site habitat	3	Fish within capture site habitat × initial habitat
Fish within capture site habitat × initial habitat	27	
Stream stage	1	
Water temperature	1	
Error	105	
Total	182	

used a descriptive approach to our observations during this event. We compared the locations of the 12 fish with functioning transmitters during the flood (16–17 January 1998) with their preflood positions (12 January 1998). We also categorized the preflood positions of fish based on the presence or absence of large woody debris and contrasted the responses to the flood of these two groups of fish.

Results

The stage of Little Jones Creek was well predicted by the stage 1 h later at the U.S. Geological Survey gage 28 km downstream on the mainstem Smith River (r^2 = 0.96). Therefore, we combined direct observations and computed predictions of stage for use as a covariate in analyses of fish movement. Stage at our gage ranged from 55 to 95 cm during our observations of radiotagged fish, except for the bankfull flood when it reached 145 cm.

Cutthroat trout captured in pools with large woody debris exhibited greater site fidelity than fish from pools lacking cover. Fish from pools with large woody debris occupied the habitats where they were captured twice as often on average as fish from pools lacking cover (81% (N = 21, SE = 5) versus 40% (N = 22, SE = 8); t test, $P < 0.01$). Similarly, the site of capture and final location were identical for 71% of fish from pools with large woody debris compared with 23% of fish from pools lacking cover. Excluding one fish from a pool without woody debris that travelled about three times further than any other fish (978 m), fish from pools without woody debris were last located an average of 90 m from their capture sites versus an average of 32 m for fish from pools with large woody debris (t test, $P < 0.01$). The relatively low site fidelity of fish captured in pools lacking cover is reflected in their greater use of large-boulder and brush habitat than fish from pools with large woody debris (Fig. 1). Fish captured in habitats lacking cover were particularly unlikely to use such habitats during the day. Indeed, fish from both types of capture sites used habitat that we classified as open more often at night than during the day: the day/night difference in the proportion of observations in open habitat differed significantly from zero for both groups (N = 21 and 22; paired t tests, $P < 0.002$ for both). The other three habitat types, which all provided cover, were occupied more often during the day than at night.

Habitat strongly influenced fish movement during the 1-day observation periods (Fig. 2). Fish initially located in open habitats at the start of an observation period travelled over longer sections of stream than did fish first located adjacent to large woody debris, while fish first located at boulders and brush moved intermediate distances ($F_{3,27}$ = 6.50, $P < 0.01$). Fish exhibited similar patterns of net movement, the difference in their locations during daytime observations on successive days ($F_{3,27}$ = 4.33, $P < 0.02$) (Fig. 2). For both total and net movement, the effect of the initial location of fish during an observation period was independent of where fish were captured ($P > 0.35$ for the interaction terms). From one day to the next, we rarely observed movement of cutthroat trout out of pools containing large woody debris. However, we commonly found fish that occupied the same habitat on successive days outside that habitat during intervening nighttime observations. Water temperature was a significant covariate with a positive slope in the analyses of both total and net movement (P = 0.03 for both response variables), while stage was not ($P > 0.25$ for both response variables). As might be predicted based on the difference in site fidelity, fish initially captured in pools with large woody debris exhibited less total movement than fish from open habitats ($F_{1,41}$ = 9.84, $P < 0.01$), although net movement was not different for fish from the two types of capture sites ($F_{1,41}$ = 2.65, P = 0.11).

During the flood, 11 of the 12 fish with functional transmitters responded with minor changes in position compared with the sites that they occupied 4 days before. One fish captured in simple habitat on 9 January 1998 was detected 70 m upstream of that capture site on 12 January 1998 and then 900 m further upstream in a tributary during the flood. Given the upstream movement by this fish prior to the flood and the behavior of other fish, we cannot confidently interpret this lengthy upstream movement as a response to the flood. Excluding this individual, during the flood, fish occupied sites an average of 17 m from those occupied 4 days before (N = 11, SE = 7). The positions of fish did not reveal a pattern in their direction of movement in response to the flood: we found five fish downstream and four upstream of their preflood positions, while two fish did not alter their longitudinal positions. Cutthroat trout apparently also moved little during the flood. Net movement of fish for the two daytime observations during the flood, on 16 and 17 January 1998, averaged only 4 m. We found seven fish in the same locations on both days, and the greatest net movement by an individual was 20 m.

The five fish that occupied pools with large woody debris on 12 January 1998 all occupied those same sections of stream during the flood. However, at a finer spatial scale, several of these fish occupied habitat during the flood that

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Fig. 1. Habitats occupied by radiotagged adult coastal cutthroat trout in Little Jones Creek, California, during fall and winter. Data are categorized by two types of capture sites: (*i*) pools formed by large woody debris (LWD) and (*ii*) pools formed by scour adjacent to bedrock that contained little or no cover for fish (open). Percentages in the top panel are based on 364 observations of 21 fish, equally divided between day and night. Percentages in the bottom panel are based on 380 observations of 22 fish, also equally divided between day and night.

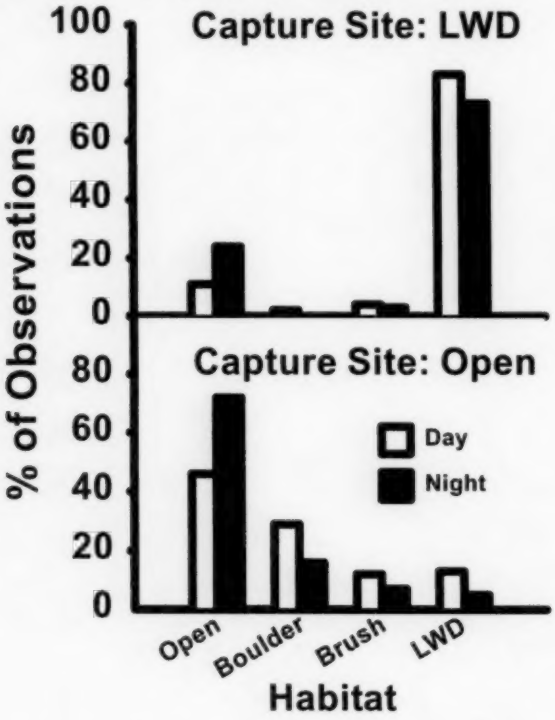
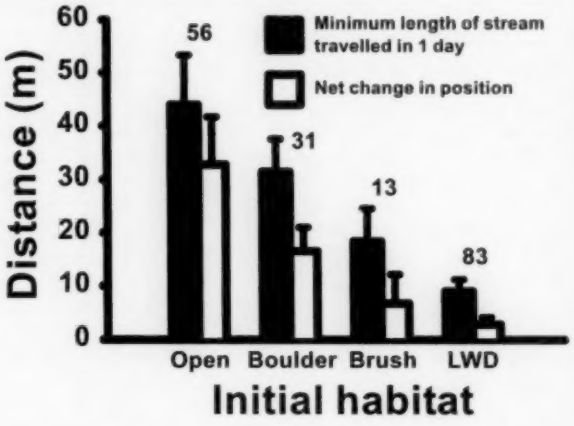


Fig. 2. Movement by radiotagged adult coastal cutthroat trout in Little Jones Creek, California, during fall and winter. Minimum stream length travelled is the distance covered by fish in 1 day, based on an initial daytime observation followed by two nighttime observations and a second daytime observation. Net change in position indicates the distance between the sites occupied by fish during two daytime observations on successive days. Numbers above the bars indicate sample size, and error bars are 1 SE; both of these reflect values for data from all fish combined. LWD, large woody debris.



they never used at lower flows. Also, none used the areas immediately downstream of large woody debris, where they were usually found at lower flows. During the flood, most areas in the main channel immediately downstream of large woody debris were sites of extreme turbulence, sometimes exacerbated by oscillations of the debris itself.

In contrast with the fish that had occupied pools with large woody debris, only two of seven fish that occupied habitats lacking large woody debris before the flood (12 January 1998) remained within those sections of stream during the flood. One of these two fish utilized habitat with low water velocity immediately below a tributary inflow, while the second occupied habitat among flooded alders near the streambank opposite the thalweg.

Overall, the 12 fish with transmitters occupied five types of habitat during the flood: (*i*) downstream of woody debris in tributaries or secondary channels (five fish), (*ii*) upstream of large woody debris in the main channel (two fish), (*iii*) open areas of low water velocity along the bank opposite the thalweg in pools formed by woody debris (two fish), (*iv*) flooded alders along the edges of straight sections of channel (two fish), and (*v*) below a tributary confluence in a pool formed adjacent to bedrock (one fish). All of these sites were along the stream margin and appeared to offer microhabitats with water velocities <15 $cm \cdot s^{-1}$, while water velocity along the thalweg during the flood was consistently >2.2 $m \cdot s^{-1}$.

Discussion

Habitat strongly influenced movement by adult cutthroat trout in Little Jones Creek during our fall–winter study period. Overall, during nonflood stream flows, fish captured at sites with large woody debris moved less than fish from pools lacking cover, and radiotagged fish found at sites with large woody debris were less mobile over 1-day observation periods independent of the type of habitat where they were captured. This lower vagility near large woody debris may simply reflect reduced movement of individuals when they encounter high-quality habitat. However, other processes appear to be involved in winter habitat selection by cutthroat trout in Little Jones Creek: extreme concentrations of fish in pools with woody debris are not apparent during moderate discharge, and a winter experiment revealed similar numbers of juvenile and adult cutthroat trout in pools lacking cover and pools where cover (including woody debris) was artificially enhanced (B.C. Harvey and J.A. Simondet, unpublished data). Perhaps lower movement rates in the vicinity of large woody debris reflect territorial defense of superior habitat by dominant individuals (Winker et al. 1995). Aggressive behavior by salmonids under winter conditions has been observed previously (Gregory and Griffith 1996).

Large woody debris and other elements providing habitat complexity appeared to be important to all the fish in this study. Cutthroat trout from pools lacking large woody debris or other cover were often found outside the specific habitats where they were captured. These fish commonly occupied higher-gradient habitats with large boulders or sites with

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large woody debris or brush, particularly during the day. The availability of large boulders appeared to be important to the fish that we captured in pools without cover. Such boulders were rare in the study reach, yet most were utilized by the relatively small number of radiotagged fish.

Our method of categorizing habitat necessarily underestimated the significance of habitat that can provide cover for cutthroat trout. Because of our inability to resolve the positions of fish on a spatial scale <1 m^2, habitat that we classified as open included areas with unembedded cobbles and small boulders. In Little Jones Creek (B.C. Harvey and J.A. Simondet, unpublished data) and elsewhere (e.g., Cunjak 1988; Griffith and Smith 1993), salmonids may conceal themselves within such substrate during the winter.

Even with our inclusion of some habitats providing cover in the open category, significant day/night differences in the use of open habitat were apparent. These differences probably reflect the diel pattern of concealment during the day and emergence at night, which has been observed in salmonids primarily during the winter (e.g., Heggenes et al. 1993; Valdimarsson et al. 1997) but also in summer (Gries et al. 1997; Gries and Juanes 1998). In Little Jones Creek, cutthroat trout apparently carry out this diel pattern both by remaining continuously in habitats providing cover and by moving from habitats providing cover during the day to habitats lacking cover, principally pools, at night.

The fitness consequences of occupying habitat with large woody debris during the winter remain unclear. Several authors have suggested that seasonal increases in risk of predation may promote greater nocturnal activity in salmonids (Fraser et al. 1993; Heggenes et al. 1993). In some areas, predation risk may be more severe in winter because swimming performance is reduced at low temperatures and predators may be more abundant. Both otters (*Lutra canadensis*) and common mergansers (*Mergus merganser*) have been observed in fall and winter, but not in summer, at Little Jones Creek, and one of the radiotagged fish in this study located in open habitat was consumed by a merganser. Occupying complex habitat and moving infrequently may reduce the risk of mortality when predation pressure is particularly severe.

Seasonal shifts in use of habitat by stream fishes, including cutthroat trout (Brown and Mackay 1995), are widely recognized. If predation risk is relatively less significant in other seasons, shifts away from large woody debris based on a balancing of predation risk and food acquisition may be common, even for fish highly ranked in dominance hierarchies. Such shifts might explain similar patterns of retention of individually tagged cutthroat trout in pools with and without large woody debris in Little Jones Creek from one summer to the next (Harvey 1998), even if pools with large woody debris provide superior habitat during part of the year. The wide variation in movement and use of habitat by cutthroat trout observed during this study may represent strategies with similar fitness consequences.

During the flood of January 1998, Little Jones Creek appeared to provide adequate habitat for adult trout over most of the study reach, even though water velocity in the thalweg consistently exceeded 2.2 $m \cdot s^{-1}$. The modest responses to flooding of cutthroat trout (this study) and other adult stream fishes (Todd and Rabeni 1989; Matheney and Rabeni 1995) provide partial explanation for minor differences between fish assemblages before and after floods (e.g., Matthews 1986; Lobón-Cerviá 1996), even though eggs and young-of-the-year fishes of some species are readily displaced and killed during flooding (e.g., Seegrist and Gard 1972; Harvey 1987). Perhaps only extreme events in relatively large channels cause significant displacement of post-young-of-the-year salmonids (Jowett and Richardson 1989). Apparently, in many settings during floods, stream fishes readily exploit habitats with relatively low water velocities along stream margins (e.g., Ross and Baker 1983; Jowett and Richardson 1994; this study).

During the flood, the majority of adult cutthroat trout that we located occupied habitat influenced by woody debris. However, fish occupied different habitats near woody debris in the main channel than they did in secondary channels and a tributary. In the smaller channels, fish occupied positions downstream of woody debris. This coincides with the observation that coho salmon occupied habitat downstream of artificial root masses during high flow in 0.9-m-wide stream channels (McMahon and Hartman 1989). In the main channel of Little Jones Creek, however, extreme turbulence downstream of large woody debris appeared to render these areas uninhabitable. Similarly, Nickelson et al. (1992*b*) hypothesized that high turbulence may have accounted for their finding that the addition of brush to plunge pools in Oregon streams did not increase the density of coho salmon, unlike the addition of brush to less-turbulent pools. When fish occupied habitat close to large woody debris in the main channel of Little Jones Creek, they were positioned upstream. Fish also occupied areas of low water velocity in the main channel along the bank opposite large woody debris. Areas of low water velocity may be relatively common during bankfull conditions in pools formed by large woody debris because woody debris may significantly deflect the flow more often than do bedrock outcrops (Lisle 1986). However, the study reach of Little Jones Creek contained several pools with large areas of low water velocity during flood conditions where bedrock did significantly deflect the flow. This observation weakens the hypothesis that cutthroat trout in the study reach would be habitat limited during floods if large woody debris were absent. Low overall movement by cutthroat trout in open habitat during the flood, and their use of the floodplain along straight sections of channel, also do not support the hypothesis that cutthroat trout are limited by the abundance of woody debris during flooding.

Overall, the extensive use of habitat associated with large woody debris, large boulders, and brush by cutthroat trout during fall and winter parallels previous studies of other salmonids (Tschaplinski and Hartman 1983; Cunjak and Power 1986). Our observations are also consistent with the hypothesis that fish assemblages in hydraulically complex stream reaches are more resistant to flooding (Pearsons et al. 1992). Finally, our observations support Cunjak's (1996) suggestion that stream habitat complexity should be maintained or restored to assure adequate winter habitat for stream fishes.

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Using stable isotopes to monitor watershed influences on aquatic trophodynamics

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Abstract: Stable C and N isotope measurements of the clam *Potamocorbula amurensis* were used to help identify watershed-level differences in food webs of San Francisco Bay. *Potamocorbula amurensis* has become widely distributed in San Francisco Bay since introduction from Asia in1986. Clam samples were collected from both the river-influenced northern arm of San Francisco Bay and the lagoonal southern arm of the Bay during 1990–1991. Carbon isotopic compositions of clams responded primarily to riverine inputs and provided an index of hydrologic mixing across the estuarine system. Nitrogen isotopic compositions of clams were more responsive to watershed nutrient loading, with higher $\delta^{15}N$ values found in South Bay, which receives stronger inputs of anthropogenic N. Routine monitoring of animal consumer isotopic compositions could be an effective way to detect long-term watershed-level changes in C and N dynamics important for secondary production in aquatic systems.

Résumé : On a utilisé des mesures des isotopes stables du C et du N présents dans le bivalve *Potamocorbula amurensis* pour établir les différences dans les réseaux alimentaires du bassin de la baie de San Francisco. *Potamocorbula amurensis* s'est largement répandu dans cette baie depuis que des spécimens asiatiques y ont été introduits en 1986. On a recueilli des échantillons de ce bivalve en 1990–1991 dans le bras nord de la baie, qui reçoit des eaux fluviales, et dans son bras sud de nature lagunaire. Les compositions en isotopes du C dans les bivalves étaient principalement tributaires des apports fluviaux, et ont fourni un indice du mélange hydrologique dans le système estuarien. Les compositions en isotopes du N dans les bivalves étaient davantage fonction de la charge en nutriants du bassin, les valeurs de $\delta^{15}N$ étant supérieures dans la partie sud de la baie, qui reçoit davantage de N anthropique. La surveillance régulière des compositions isotopiques des consommateurs pourrait être un moyen efficace pour détecter les changements à long terme qui se produisent à l'échelle des bassins dans la dynamique du C et de N et qui sont importants pour la production secondaire dans les systèmes aquatiques.

[Traduit par la Rédaction]

Introduction

Stable isotope measurements are now used in many ways in studies of animal ecology, e.g., to describe major flow pathways of organic matter in food webs, to follow animal movements and migrations from one food web to another, and to show variations in feeding strategies among individual animals (DeNiro and Epstein 1978, 1981; Fry et al. 1978; Schell et al. 1989; Trust 1993; Gannes et al. 1997). Another way to use stable isotopes is to monitor watershed-level processes such as inputs of terrestrial organic material to aquatic systems. For example, C isotope studies of sediments have been widely used to calculate terrestrial inputs to both freshwater and marine systems (Fry and Sherr 1984).

Stable isotope monitoring of watershed-level influences on trophic dynamics in aquatic systems is also possible. This monitoring differs from sediment work in that the focus is on organic material that is labile and actively processed in aquatic food webs rather than on the refractory material accumulating in sediments. Most methods of monitoring aquatic secondary production are quite labor-intensive (e.g., repeated trawl or benthic sampling surveys, Dara and Clarke 1998), and simpler methods that can help show linkages between watershed-level inputs and responses of aquatic food webs seem desirable. This study investigated whether isotope distributions in a clam (*Potamocorbula amurensis*) collected across a large estuary might provide an integrated picture of seasonal and spatial differences in organic matter supporting estuarine food webs.

Potamocorbula amurensis appeared in San Francisco Bay from Asia in 1986, probably introduced in ballast water (Carlton et al. 1990). The small clam quickly spread throughout the estuary and became a dominant component of the benthos, colonizing sediments throughout the full salinity range. The clams feed when sitting on top of the sediment surface and can consume both phytoplankton and bacteria (Cole et al. 1992; Werner and Hollibaugh 1993).

The data used in this paper were collected by Cloern et al. (1993) as part of ongoing studies of San Francisco Bay and its watersheds by the U.S. Geological Survey and associated scientists. The data have been previously evaluated with respect to lipid biomarker and C isotopic compositions (Canuel et al. 1995; Cloern 1996). This study reanalyzes those published C isotopic results and includes an emphasis on the N isotopic compositions ($\delta^{15}N$ values) that were measured as part of the original study (Cloern et al. 1993) but not reported in subsequent publications.

North Bay and South Bay are the two major subestuaries of San Francisco Bay and differ in hydrology and in nutrient loading. Riverine inflows from the Sacramento and San Joaquin rivers in northern San Francisco Bay are seasonally

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Fig. 1. Map of San Francisco Bay showing sampling locations for *P. amurensis*, April 1990 – April 1991. North Bay extends across San Pablo Bay to the delta region at the confluence of the San Joaquin and Sacramento rivers. For each collection site (circles), average clam $\delta^{13}C$ (negative values) and $\delta^{15}N$ (positive values) are shown.

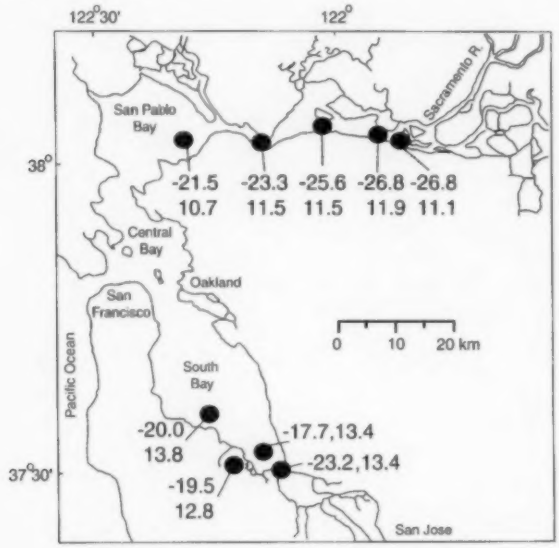
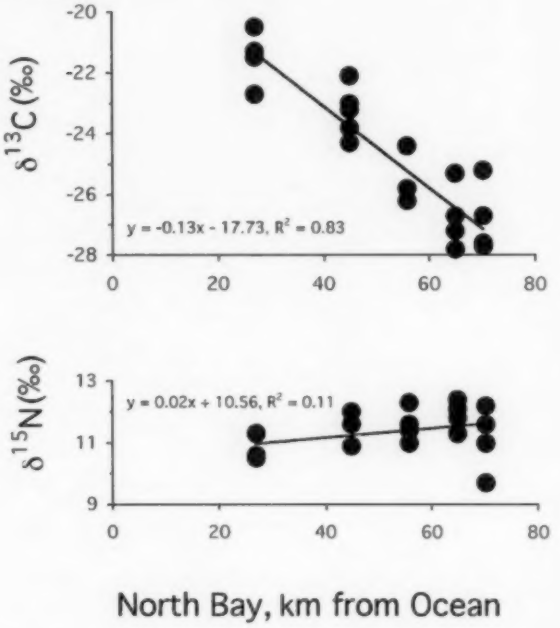


Fig. 2. C and N isotopic compositions of North Bay clams versus distance from the mouth of the estuary at the Golden Gate Bridge.



strong and introduce wastewater and agricultural nutrients from Sacramento and the Central Valley of California. Dissolved inorganic N (DIN) levels can average near 15 μM in these rivers (Rast and Sutton 1988; Hager and Schemel 1992). South Bay is much less river influenced than North Bay and is lagoonal in character. South Bay receives wastewater effluents from more local sources, including several sewage treatment facilities, and DIN levels are often in the 50–100 μM range (Hager and Schemel 1992). This study builds on the previous analyses by Cloern et al. (1993) and Canuel et al. (1995) to test how and when stable isotope compositions of clams record watershed-level differences in hydrology and nutrient loading for North Bay versus South Bay.

Materials and methods

Sample collection occurred along bay-wide transects from North Bay to South Bay (Fig. 1). Seston was collected on filters from January 1990 to April 1992, while clams were collected from April 1990 to April 1991 (Cloern et al. 1993). Guts were removed from clams at the time of collection and remaining organic tissues composited from three to five animals per site, lyophilized, and used for isotopic analyses. Processing of seston samples for particulate organic C and N has been described previously (Cloern et al. 1993; Canuel et al. 1995). Dried samples were measured for $\delta^{13}C$ and $\delta^{15}N$ using CO_2 and N_2 gas produced in sealed tube combustions (Rau et al. 1990).

Results

Isotopic compositions of clams were distinct in the two arms of San Francisco Bay (Fig. 1). These between-watershed differences (South Bay versus North Bay) were largely due to higher $\delta^{15}N$ values in clams that were collected from South Bay (Figs 1, 2, and 3). Between-year differences were evident for both C and N isotopes in the South Bay clam collections, while clam samples from North Bay yielded generally similar results in the two collection years (Fig. 3).

In North Bay, C isotopic compositions increased regularly from –27 to –21‰ along a 46-km transect from the freshwater end of the estuary to the more marine San Pablo Bay (Figs. 1 and 2). Average clam values of –26.8 to –25.6‰ at the uppermost three stations were not significantly different from one another (t test, $p > 0.05$), while the down-estuary –23.3 and –21.5‰ mean values were each significantly different from other North Bay mean values (t test, $p < 0.05$). The N isotopic compositions of clams also changed across North Bay, trending from higher values near 12‰ at the freshwater end of the estuary to values of 10.7‰ in San Pablo Bay (Figs. 1 and 2). Average $\delta^{15}N$ values were generally not statistically different (t test, $p > 0.05$) from one another across the transect (Fig. 1), with the exception that the 10.7‰ value found furthest down-estuary was significantly different (t test, $p < 0.05$) from the midtransect 11.5–11.9‰ mean values. Overall, there was a declining trend in $\delta^{15}N$ down-estuary in North Bay that was associated with increasing C isotope values (Figs. 2 and 3).

The seston collected at the freshwater end of North Bay between January 1990 and February 1992 in 15 low-salinity (<1‰) samples averaged (±95% confidence limits) –27.0 ± 0.4‰ $\delta^{13}C$ and 3.8 ± 0.6‰ $\delta^{15}N$. The seston $\delta^{13}C$ values were quite similar to those of the –27‰ $\delta^{13}C$ values of clams collected in this region, but the seston $\delta^{15}N$ values were much lower than the clam 12‰ $\delta^{15}N$ values.

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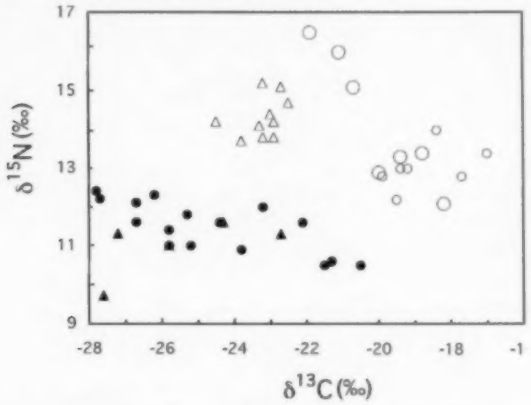
Fig. 3. Isotope values for *P. amurensis* collected in North Bay (solid symbols) and South Bay (open symbols). Samples were collected in 1990 (circles) and 1991 (triangles). Large open circles denote South Bay samples collected in the summer and winter between the two spring bloom periods of 1990 (small open circles) and 1991 (open triangles).



In the South Bay lagoonal system, most samples were collected in spring 1990 and spring 1991 over a fairly close-spaced set of stations in the lower, central part of the lagoon (16 km maximum between collection stations, Fig. 1). There were significant differences (t test, $p < 0.05$) in both C and N isotopic compositions between the two spring periods, with mean values (±95% confidence limits) of –18.7 ± 0.8 versus –23.2 ± 0.4‰ for $\delta^{13}C$ in 1990 versus 1991 and 13.0 ± 0.4 versus 14.3 ± 0.3‰ for $\delta^{15}N$ in 1990 versus 1991. Clams collected between the two spring bloom periods had values similar to those of the 1990 spring collections or intermediate values (see Fig. 3, large open circles).

Discussion

Stable isotopic measurements of clam tissues showed watershed-level differences in samples from North Bay versus South Bay that could be related to differences in hydrology and nutrient loading. Hydrologic effects were more evident in the C isotope data, while nutrient effects were more evident in the N isotope data.

The C isotopic compositions of the *P. amurensis* collections have been discussed by Canuel et al. (1995) in terms of clams feeding on planktonic materials. Canuel et al. (1995) showed that in San Francisco Bay, C isotopic compositions of phytoplankton and clams vary in concert with those of dissolved inorganic C (DIC), suggesting that plankton use DIC with a roughly constant isotopic fractionation during photosynthetic C uptake and that plankton provide most of the nutrition for the clams. Biomarker analyses of lipids supported the view that *Potamocorbula* selectively utilizes planktonic foods in this estuary (Canuel et al. 1995). Because freshwater inflows affect salinity and mixing ratios of marine and freshwater DIC in San Francisco Bay (Spiker 1980), $\delta^{13}C$ values of DIC, plankton, and clams record hydrologic mixing dynamics across San Francisco Bay. The highest clam and seston $\delta^{13}C$ values were observed in the 1990 South Bay samples in conjunction with a strong diatom bloom (Canuel et al. 1995), consistent with other observations of high seston and consumer $\delta^{13}C$ values in diatom-rich environments (Fry and Wainright 1991; Fry 1996). Lower $\delta^{13}C$ values were found in the 1991 spring samples from South Bay (Canuel et al. 1995) (Fig. 3), a time when stormy and flooding conditions prevailed in South Bay (Canuel et al. 1995; J. Cloern, personal communication). Storms may have introduced ^{13}C-depleted DIC into the water column from runoff or from porewater released during sediment resuspension events, with lower $DI^{13}C$ values leading to the observed lower seston and clam $\delta^{13}C$ values.

The clam $\delta^{15}N$ values in San Francisco Bay are quite high relative to the 0–8‰ $\delta^{15}N$ values observed for invertebrates in pristine freshwater and marine systems (Minagawa and Wada 1984; Fry 1988, 1991; Meili et al. 1993; Zanden et al. 1997) and likely indicate an important role for anthropogenic N loading in this estuary. Several studies show high $\delta^{15}N$ values of sediments, algae, invertebrates, and fish in lakes and estuaries where inputs of wastewater N are large (Hobbie et al. 1990; Cabana and Rasmussen 1996; Wainright et al. 1996; Hansson et al. 1997; McClelland et al. 1997; Voss and Struck 1997). High $\delta^{15}N$ values in ammonium and nitrate from wastewater sources seem responsible for $\delta^{15}N$ labeling of entire estuaries (McClelland and Valiela 1998), although the actual isotopic compositions of ammonium and nitrate have not been measured regularly across impacted sites to verify this assumption. Wastewater loading to South Bay is known to be strong and the very high 12–17‰ $\delta^{15}N$ values for clams in this area probably reflect this strong local loading. For North Bay, riverine inputs are seasonally strong, and both the San Joaquin and Sacramento rivers contain substantial wastewater N loadings (Hagar and Schemel 1992). The North Bay pattern of highest clam $\delta^{15}N$ values farthest upstream and only slightly lower $\delta^{15}N$ values downstream may indicate that wastewater N is not only important for plankton production in upriver portions of the estuary, but also important far down the estuary to San Pablo Bay, the most downstream region sampled in this study. Further sampling in Central Bay and in the nearshore ocean offshore of this estuary will be necessary to carefully define the spatial extent of anthropogenic N influence in this estuary.

The $\delta^{15}N$ values of clams at the freshwater end of North Bay deserve special discussion, for there was a large 8‰ discrepancy between $\delta^{15}N$ values of bulk seston (3.8 ‰) and clams (12‰) in this area. Rast and Sutton (1988) found a similar large $\delta^{15}N$ discrepancy in this part of San Francisco Bay in the summer of 1985, when seston values averaged about 5‰ and large zooplankton (mysids and zooplankton >154 m) values ranged from 9 to 14‰. There are several possible explanations for the discrepancy between filter-feeder and seston $\delta^{15}N$ values: (*i*) there are intermediate trophic linkages in a microbial loop/microzooplankton food web leading from riverine detrital material to clams, and since extra trophic steps in planktonic food webs lead to increasing ^{15}N enrichment (Meili et al. 1993; Fry and Quinones 1994), higher $\delta^{15}N$ values are observed in filter-feeders, (*ii*) bacteria that colonize detrital riverine material immobilize ^{15}N-enriched N from the water column (Caraco et al. 1998), and filter-feeders consuming these bacteria have higher $\delta^{15}N$ values, and (*iii*) riverine seston is largely terrestrial and refractory to food web use (Canuel et al. 1995), but the large quantity of this material masks a relatively minor

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^{15}N-enriched riverine/estuarine phytoplankton component selectively used by filter-feeders.

It is difficult to distinguish between these three reasonable alternatives without direct δ^{15}N determinations of nutrients, phytoplankton, bacteria, and microzooplankton. Isotopic methodology is advancing so that measuring isotopic compositions of these N pools is becoming more feasible (Coffin et al. 1994; Sachs 1997; Sigman et al. 1997). Future research utilizing these advances may more clearly show how changes in foods result in the observed clam and zooplankton isotope values. Comparative studies with other filter-feeders (barnacles, oysters, mussels, sponges, bryozoans, etc.) should also help focus the search for the best isotope trophic biomonitor ("trophometer") for free-living bacteria versus phytoplankton versus detritus.

Long-term monitoring of stable isotopic compositions of filter-feeders has already begun in San Francisco Bay (Rast and Sutton 1988; Cloern et al. 1993; Canuel et al. 1995; this study) and in other systems where animals have been regularly sampled along salinity or river–ocean transects (e.g., Incze et al. 1982; Stephenson and Lyon 1982; Fry 1984; Peterson et al. 1985; Simenstad and Wissmar 1985; Ruckelshaus et al. 1993; Mitchell et al. 1996; Deegan and Garritt 1997; Riera and Richard 1997; Riera 1998). Developing more regular stable isotope transect studies as monitoring tools should help assess how watershed-level changes in hydrology and nutrient inputs affect long-term development of aquatic ecosystems and their food webs. Restoration ecology goals for aquatic systems might also include restoration of baseline isotopic compositions for target organisms such as filter-feeders because isotopic compositions of animal consumers are robust integrative measures of organic matter dynamics in aquatic systems (Cabana and Rasmussen 1996). Including stable isotope assays in routine sampling programs may prove an efficient way of monitoring watershed-level effects on trophodynamics in downstream aquatic systems.

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Effects of the spawning migration on the nutritional status of anadromous Atlantic salmon (*Salmo salar*): insights from stable-isotope analysis

R.R. Doucett, R.K. Booth, G. Power, and R.S. McKinley

Abstract: Anadromous Atlantic salmon (*Salmo salar*) were collected during their spawning migration along the Exploits River, Newfoundland, in 1996 and analyzed for stable-isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and tissue concentrations of protein and lipid. Fish became progressively more ^{13}C enriched during the spawning migration (about –24 to –19‰), and the degree of enrichment was dependent on tissue type (red muscle: +4.1‰, liver: +2.6‰, white muscle: +1.3‰). Only liver showed consistent changes in $\delta^{15}N$, as overwintering kelts (13.5 ± 0.8‰) were about +2‰ more enriched than upstream migrants (11.4 ± 1.5‰). Isotopic enrichment in migrating salmon appeared to result from mobilization, reorganization, and catabolism of stored lipid and protein reserves associated with the cessation of feeding upon entering freshwater. The most significant correlations existed between lipid content and $\delta^{13}C$ in red muscle (r^2 = 0.67) and protein content and $\delta^{15}N$ in liver (r^2 = 0.32). This study shows that fasting affects the stable-isotope ratios of both carbon and nitrogen in anadromous fishes and that nutritional status should be considered when inferring food web relationships from the isotopic compositions of migrating salmonids.

Résumé : On a recueilli des saumons atlantiques (*Salmo salar*) anadromes durant leur migration de fraye dans la rivière des Exploits, à Terre-Neuve, puis établi les ratios des isotopes stables ($\delta^{13}C$ et $\delta^{15}N$) et les concentrations tissulaires des protéines et des lipides. Les poissons renfermaient de plus en plus de ^{13}C au fur et à mesure que progressait leur migration de fraye (environ –24 à –19‰), et l'importance de cet accroissement dépendait du type de tissu (muscle rouge : +4,1‰, foie : +2,6‰, muscle blanc : +1,3‰). Ce n'est que dans le foie qu'on a observé des variations régulières de $\delta^{15}N$, celui des charognards hivernants (13,5 ± 0,8‰) en renfermant environ +2‰ de plus que celui des saumons en montaison (11,4 ± 1,5‰). L'enrichissement isotopique chez les saumons en migration a semblé résulter d'une mobilisation, d'une réorganisation et du catabolisme de leurs réserves de lipides et de protéines, associés à l'arrêt de leur alimentation dès leur entrée en eau douce. On a observé les corrélations les plus significatives entre la teneur en lipides et $\delta^{13}C$ dans le muscle rouge (r^2 = 0,67) et entre la teneur en protéines et $\delta^{15}N$ dans le foie (r^2 = 0,32). Cette étude montre que le jeûne influe sur les ratios des isotopes stables du carbone et de l'azote chez les poissons anadromes et que l'état nutritionnel devrait être pris en considération quand on infère des relations trophiques à partir des compositions isotopiques des salmonidés migrateurs.

[Traduit par la Rédaction]

Introduction

Most Atlantic salmon (*Salmo salar*) are anadromous and undergo extensive migrations between marine feeding areas and freshwater spawning and rearing sites. During the upstream spawning migration, salmon undergo morphological changes and gonadal maturation (Mills 1989). They must also adjust to changes in salinity, water temperature fluctuations, and swimming against variable and sometimes rapid currents. These stressors induce increased metabolic demands on salmon at a time when they also cease feeding (Kadri et al. 1995). Thus, all energy requirements associated with the spawning migration must be met by stored reserves (Jonsson et al. 1991).

The main storage products used by salmonids are lipids and proteins (Higgs et al. 1995). Lipids, specifically free fatty acids (FFAs), provide the aerobic energy needed by red muscle to propel fish during sustained locomotion (Love 1980), and reductions in plasma nonesterified FFAs have been observed in migrating salmon (Ballantyne et al. 1996). Many salmonids also possess the ability to catabolize proteins during gluconeogenesis (Suarez and Mommsen 1987). When necessary, proteins and amino acids can be extracted from white muscle (Mommsen et al. 1980), and depending

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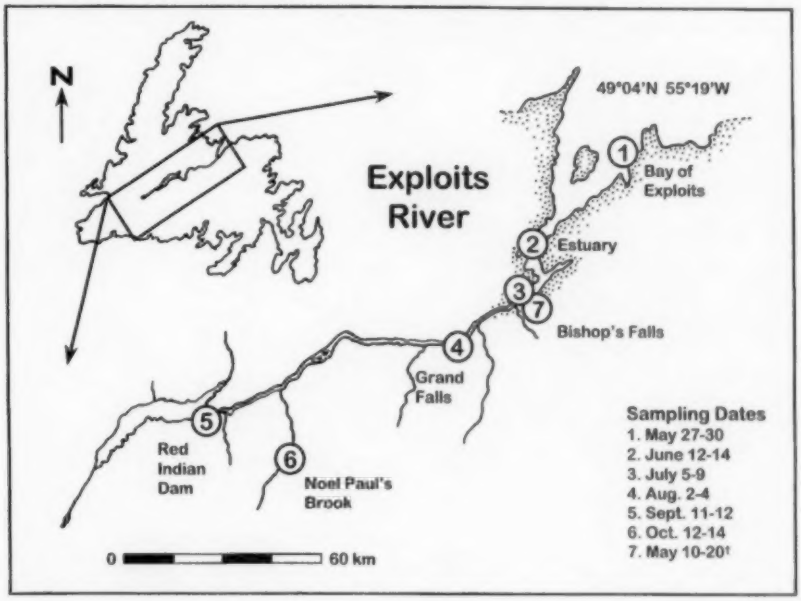
Fig. 1. Map of the study area showing the location of collection sites along the Exploits River, Newfoundland, and the sampling dates when adult Atlantic salmon were caught. The dagger denotes that kelts were collected in May 1996 (we assumed that kelts from the 1995 spawning population were not isotopically distinct from those in 1996).



on the degree of utilization, this may contribute to declines in swimming performance in migrating salmon and influence survival in postspawning kelts (Booth 1998).

Stable-isotope analysis might provide additional information on nutritional stress in migrating salmon. Stable-isotope analysis has been used previously to interpret diet and trophic position for many anadromous salmonids (e.g., Mathisen et al. 1988; Kline et al. 1990, 1993; Bilby et al. 1996; Doucett et al. 1999*a*, 1999*b*), including Atlantic salmon (Doucett et al. 1996). The method is based on the fact that naturally occurring stable-isotope ratios of common elements such as carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) are assimilated into primary producers with signatures characteristic of various biogeochemical processes and are passed along food chains with relatively predictable change (e.g., Peterson and Fry 1987; Rundel et al. 1989; Lajtha and Michener 1994; and references contained therein). Animal $\delta^{13}C$ remains relatively unaffected by trophic transfer, whereas predictable enrichment occurs in $\delta^{15}N$ from one trophic position to the next. Typically, at each trophic transfer of food energy, $\delta^{13}C$ values enrich by 0–1‰, whereas $\delta^{15}N$ values increase by 3–5‰ (DeNiro and Epstein 1978; Minagawa and Wada 1984; Hobson and Clark 1992; but see Focken and Becker 1998). When multiple tissues are measured, $\delta^{13}C$ and $\delta^{15}N$ may provide both short- and long-term dietary information because tissues often have different elemental turnover rates (Tieszen et al. 1983). However, tissue-specific isotope turnover rates have not been identified in fish, and isotopic responses to changes in food could take years in slow-growing wild populations (Hesslein et al. 1993).

Fasting animals tend to have stable-isotope ratios that are distinct from those fed ad libitum. Hobson et al. (1993) found ^{15}N enrichment in the tissues of starved laboratory-raised Japanese quail (*Coturnix japonica*) and wild Ross' geese (*Chen rossi*). The increase in ^{15}N content was believed to be associated with the catabolism of body protein, which, through the processes of deamination and transamination, provides more ^{15}N-enriched substrates for protein synthesis than those of assimilated foods (Gaebler et al. 1966). Similar enrichment would be expected in the $\delta^{13}C$ signature of animal tissues as a result of processing body lipids during nutritional stress because lipids are more ^{13}C depleted than other tissue components (DeNiro and Epstein 1977) and metabolism of storage lipids would presumably result in ^{13}C enrichment in the remaining tissue pool (Tieszen et al. 1983).

In this study, we used $\delta^{13}C$ and $\delta^{15}N$ values to investigate the effects of the freshwater upstream spawning migration and subsequent overwintering period on the nutritional status of anadromous Atlantic salmon in the Exploits River, Newfoundland (Fig. 1). We refer to Atlantic salmon as "upstream migrants" as they move from the sea to the riverine spawning areas (sites 1–5), "mature fish" when they have arrived at the spawning site and are ready to spawn (site 6), and "kelts" if they have survived spawning and have overwintered in freshwater (site 7). It was our assumption that, as salmon penetrated farther upstream, they would have spent more time in freshwater without feeding. Consequently, changes in stable-isotope ratios of their tissues could be used to monitor depletion of their stored energy reserves. Enrichment in both $\delta^{13}C$ and $\delta^{15}N$ would indicate that both lipid and protein were being mobilized as energy reserves, and the degree of isotopic enrichment in carbon and nitrogen could be used to indicate the relative importance of each reserve during the migration period. Effects of metabolic stress on stable-isotope ratios have been studied previously in mammals (Ambrose and DeNiro 1987) and birds

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Table 1. Size distribution and sample size (*N*) of adult Atlantic salmon collected during their spawning migration along the Exploits River, Newfoundland, in 1996.

Site	Fork length (cm)	Mass (kg)	*N*
1. Bay of Exploits	57.1±4.1	2.0±0.2	6
2. Estuary	52.6±2.8	1.8±0.2	8
3. Bishop's Falls	54.1±4.4	1.8±0.3	7
4. Grand Falls	56.7±1.8	1.8±0.2	8
5. Red Indian Dam	60.0±7.7	1.9±0.8	7
6. Noel Paul's Brook	56.5±3.0	1.8±0.4	8
7. Estuary (kelts)	54.4±7.0	0.9±0.4	8

Note: All values for fork length and mass are expressed as means ± 1 SD.

(Hobson et al. 1993). Coincidentally, Kline et al. (1993) first reported isotopic differences between fresh and spawned-out sockeye salmon (*Oncorhynchus nerka*) in Alaska, but to our knowledge, this is the first study to measure $\delta^{13}C$ and $\delta^{15}N$ in an anadromous fish during its spawning migration and to correlate potential changes in stable-isotope ratios with nutritional information.

Methods and materials

Study site and sample collection

Atlantic salmon were collected over a 5-month period, between May and October 1996, as they migrated up the Exploits River, Newfoundland (Fig. 1). Numbers of migrating fish were distributed unimodaly over a 3- to 4-week interval. Sampling was timed to coincide with peak numbers at each site, as determined by Department of Fisheries and Oceans personnel monitoring the migration. The Exploits River is a complex system with several tributaries and the mainstem supporting Atlantic salmon production. Since the late 1960s, the river has been modified by fishway construction and manipulated by fry planting and adult transfers to enhance Atlantic salmon production (O'Connell and Bourgeois 1987; Randall et al. 1989). The resulting anadromous run is dealt with as a single unit for management purposes, and we based our study on the assumption that we were sampling the same population as it migrated upriver. In the interests of conservation, sample sizes had to be kept small and comprised six to eight randomly selected fish captured at each site using tended gill nets (sites 1 and 2) or fixed traps (sites 3–7). Of 52 adult Atlantic salmon collected for this study, 30 (58%) were female and 22 (42%) were male. Differences in fork length existed (analysis of variance (ANOVA), $P < 0.001$), but there was no consistent trend among sites (Table 1), and almost all salmon (>90%) were one-sea-winter fish (i.e., salmon that had spent only one winter feeding at sea). No differences in body mass were found, with the exception that kelts weighed about 50% less than upstream migrants and mature fish (Tukey's HSD, $P < 0.001$).

Red and white muscle and liver tissues were used in the analyses. White muscle was sampled dorsal to the lateral line and anterior to the dorsal fin. Red muscle was removed from a region proximal to the lateral line but also anterior to the dorsal fin. These two types of muscle were selected for analyses because of their different roles in fish movement and energy utilization (Bone 1978). White muscle is used mostly for anaerobic locomotion and relies on glycogen as a substrate for energy metabolism. Red muscle is an aerobic tissue that stores lipids extracellularly for quick mobilization of FFAs during sustained swimming activity. Liver was sampled because it was expected to have a faster turnover rate than muscle (Tieszen et al. 1983; but see Hesslein et al. 1993), and we suspected that effects of nutritional stress might be most apparent in this tissue.

Lipid and protein content

To assess losses of stored reserves due to nutritional stress, tissue concentrations of protein and lipid were determined for individual fish from each site. Protein content in muscle and liver homogenates was estimated using a modification of the Lowry method (Polachek and Cabib 1981). Lipid content was measured using the extraction procedure of Bligh and Dyer (1959). All lipid and protein levels were recorded as mean ± 1 SD milligrams per gram wet mass for each specific tissue.

Stable-isotope analysis

To determine the $\delta^{13}C$ and $\delta^{15}N$ values of individual fish, about 1 mg of pulverized, freeze-dried tissue samples was weighed into small tin boats and combusted in a Carlo-Erba elemental analyzer. Purified CO_2 and N_2 were subsequently released into a continuous-flow isotope ratio mass spectrometer (CFIRMS) (Micromass, Manchester, U.K). All isotopic analyses were performed on non-lipid-extracted samples, as it was our direct intention to measure the utilization of lipid over the migration period.

Stable-isotope ratios are expressed in delta (δ) notation, in per mill (‰) units, relative to international standards according to the following formula:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where δX is either $\delta^{13}C$ or $\delta^{15}N$ and R is either $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Isotopically "enriched" samples contain relatively more of the heavier isotopes, while isotopically "depleted" samples contain relatively less ^{13}C or ^{15}N. International standards are VPDB carbonate (Coplen 1996) and atmospheric nitrogen (Mariotti 1983) and are by definition set at 0‰. Replicate samples of universally available standards were reasonably precise (IAEA-CH6: −10.49 ± 0.19‰ (mean ± 1 SD), $N = 51$; IAEA-N2: 20.23 ± 0.14‰, $N = 48$) and did not differ statistically from expected values (one-sample *t*-test, $P > 0.05$).

Statistical analyses

ANOVA was used to test for significant differences among sites. Tukey's HSD test was then applied a posteriori to identify differences between specific sites (Sokal and Rohlf 1995). We used model 2 linear regression to determine the significance of relationships between $\delta^{13}C$ and lipid content and between $\delta^{15}N$ and protein content. Analysis of covariance (ANCOVA) was employed to test for differences in regression slopes between female and male Atlantic salmon. Assumptions of all parametric tests were checked using graphical analysis of standardized residuals. All analyses were performed using SYSTAT (version 7.0, SPSS Inc., Chicago, Ill.). The maximal type 1 error rate was set at $\alpha = 0.05$.

Results

Lipid and protein content

Lipid content decreased in Atlantic salmon during the spawning migration (Fig. 2*a*). This observation was most evident in red muscle (ANOVA, $P < 0.001$) where lipid reserves were reduced from a high of 89.4 ± 9.8 $mg \cdot g^{-1}$ in upstream migrants to 40.9 ± 10.6 $mg \cdot g^{-1}$ in mature fish and finally to 12.4 ± 1.2 $mg \cdot g^{-1}$ in kelts (Tukey's HSD, $P < 0.01$). Lipid stores also declined in liver tissue (ANOVA, $P < 0.001$), but decreases were slight in comparison with those in red muscle (upstream migrants: 21.1 ± 2.5 $mg \cdot g^{-1}$, mature fish: 16.3 ± 4.0 $mg \cdot g^{-1}$, kelts: 12.2 ± 2.3 $mg \cdot g^{-1}$) (Tukey's HSD, $P < 0.05$). No significant differences in lipid composition were observed in white muscle over the migration period (ANOVA, $P > 0.05$).

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Fig. 2. (*a*) Lipid and (*b*) protein content in red and white muscle and liver tissue in adult Atlantic salmon collected during their spawning migration along the Exploits River, Newfoundland, in 1996. The broken vertical line depicts the site where spawning occurred and separates the graph into a prespawning upstream migration section on the left and an overwintering stage on the right. All values are shown as means ± 1 SD. Note that no data were available for fish collected at site 1 (Bay of Exploits).

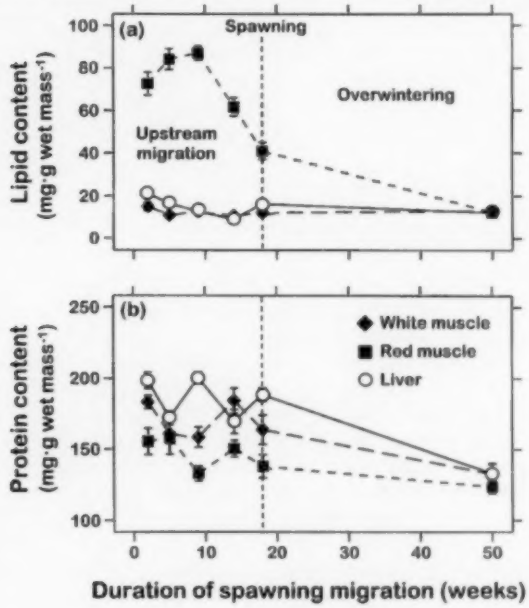
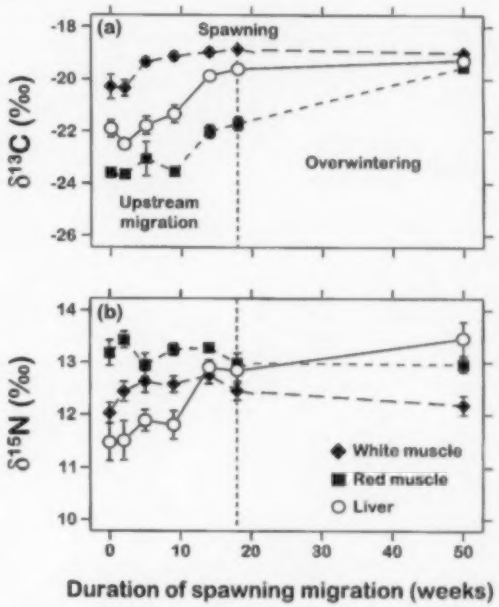


Fig. 3. Stable-isotope ratios of (*a*) carbon and (*b*) nitrogen in red and white muscle and liver tissue in adult Atlantic salmon collected during their spawning migration along the Exploits River, Newfoundland, in 1996. The broken vertical line depicts the site where spawning occurred and separates the graph into a prespawning upstream migration section on the left and an overwintering stage on the right. All values are shown as means ± 1 SD.



Protein levels were more variable than lipid content in migrating salmon, but significant differences still existed among sites (ANOVA, $P < 0.01$) (Fig. 2*b*). Although protein levels in upstream migrants and mature fish were not distinct (Tukey's HSD, $P > 0.05$), kelts had consistently lower values than either group (Tukey's HSD, $P < 0.05$). Protein losses were greatest in liver tissue (upstream migrants, 198.4 ± 15.2 mg·g^{-1}; mature fish, 188.4 ± 12.3 mg·g^{-1}; kelts, 133.3 ± 15.8 mg·g^{-1}) and white muscle (upstream migrants, 183.3 ± 11.8 mg·g^{-1}; mature fish, 163.8 ± 26.9 mg·g^{-1}; kelts, 132.9 ± 19.8 mg·g^{-1}). Red muscle also showed reductions in protein levels, but losses were small (i.e., < 20%) and were marginally significant only between fish at sites 2 and 7 (Tukey's HSD, $P = 0.023$).

$\delta^{13}C$ and $\delta^{15}N$ values

All tissues showed changes in $\delta^{13}C$ values from the time that fish left salt water to the overwintering kelt stage (Fig. 3*a*). In order of magnitude, significant ^{13}C enrichment was observed in red muscle > liver > white muscle (+4.1 > +2.6 > +1.3‰, respectively) from site 1 to site 7 (ANOVA, $P < 0.001$). This ^{13}C enrichment was most likely due to continued reductions in lipid levels over the migration period, as nonextracted samples eventually reached the same $\delta^{13}C$ values as lipid-extracted samples (Fig. 4). Most of the ^{13}C enrichment occurred between upstream migrants and mature fish, and only red muscle showed a significant difference between mature fish (–21.7 ± 0.7‰) and kelts (–19.5 ± 0.3‰) (Tukey's HSD, $P < 0.001$). Interestingly, all sampled tissues converged on the same final $\delta^{13}C$ value (i.e., about –19‰) in kelts.

Enrichment in $\delta^{15}N$ was generally less than that observed in $\delta^{13}C$ for Atlantic salmon over the spawning migration (Fig. 3*b*). No differences in $\delta^{15}N$ were seen in either red or white muscle (ANOVA, $P > 0.05$). However, liver was significantly more ^{15}N enriched in kelts (site 7: $\delta^{15}N$ = 13.5 ± 0.8‰) than in mature fish (site 6: $\delta^{15}N$ = 12.8 ± 0.6‰) or in upstream migrants (site 1: $\delta^{15}N$ = 11.4 ± 1.5‰) (Tukey's HSD, $P < 0.05$).

Stable-isotope ratios were correlated with protein and lipid content, and these results differed between the sexes (Table 2; Fig. 5). Of 12 model 2 regression equations, six were statistically significant ($P < 0.05$), and four of the six were for female salmon. The strongest relationships were observed between red muscle $\delta^{13}C$ and lipid content ($P < 0.001$, both sexes) and liver $\delta^{15}N$ and protein composition ($P < 0.001$, females only). Because there was no significant interaction between lipid or protein levels and sex (ANCOVA, $P > 0.05$), males and females were pooled to determine the relationship between stable-isotope ratios and nutritional status in migrating Atlantic salmon. Analyses on pooled data found significant ^{13}C enrichment in red muscle with reduction in lipid content (Fig. 5*a*) (model 2 regression, $P < 0.001$) and significant ^{15}N enrichment in liver tissue associated with protein loss (Fig. 5*b*) (model 2 regression, $P < 0.001$).

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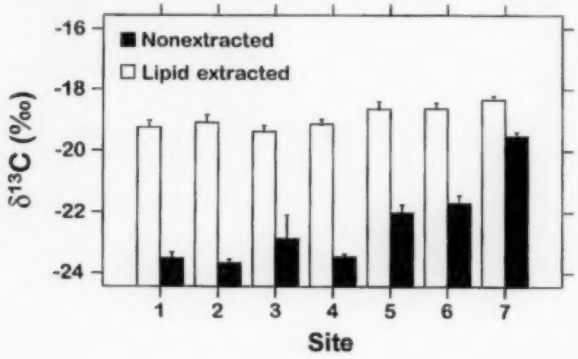
Table 2. Relationships between stable-isotope ratios of carbon (C13) and nitrogen (N15) and lipid (*L*) and protein (*P*) content (mg·g tissue wet mass^{-1}), respectively, in liver (l) and red (r) and white muscle (w) of female and male adult Atlantic salmon migrating along the Exploits River, Newfoundland, in 1996.

Tissue	Dependent	Independent	Sex	r^2	df	Equation
Liver	$\delta^{13}C$	Lipid	Female	0.24**	26	$C13_l = -0.14L_l - 18.64$
			Male	0.03 ns	14	
	$\delta^{15}N$	Protein	Female	0.37***	26	$N15_l = -0.03P_l + 18.00$
			Male	0.21 ns	14	
Red muscle	$\delta^{13}C$	Lipid	Female	0.78***	25	$C13_r = -0.05L_r - 19.46$
			Male	0.52***	17	$C13_r = -0.04L_r - 19.76$
	$\delta^{15}N$	Protein	Female	<0.01 ns	25	
			Male	0.33*	17	$N15_r = 0.01P_r + 11.42$
White muscle	$\delta^{13}C$	Lipid	Female	0.20*	26	$C13_w = -0.09L_w - 18.15$
			Male	<0.01 ns	18	
	$\delta^{15}N$	Protein	Female	0.02 ns	26	
			Male	0.18 ns	18	

Note: Coefficients of determination (r^2), significance levels (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, $P > 0.05$), and degrees of freedom (df) are given.

Fig. 4. Comparison of lipid-extracted and nonextracted $\delta^{13}C$ values in the red muscle of adult Atlantic salmon collected from seven sites along the Exploits River, Newfoundland, in 1996. All values are shown as means ± 1 SD. Lipid-extracted samples were obtained using a 2:1 (v/v) chloroform–methanol solvent and the extraction procedure of Bligh and Dyer (1959).



Discussion

Lipid content and $\delta^{13}C$

Overall, we observed significant effects of the spawning migration on the nutritional status and stable-isotope composition of anadromous Atlantic salmon in the Exploits River, Newfoundland. Changes were most obvious in lipids, which, in red muscle, demonstrated a 75% reduction in content over the entire study period, although mostly during upstream migration (Fig. 2*a*). Unlike red muscle, little or no reduction in lipid content was observed in liver and white muscle, and losses were restricted only to upstream migrants. Red muscle provides the primary locomotory force for sustained swimming activity in migrating fish (Bone 1978) and mobilizes FFAs from extracellular pools as required. Reductions in lipid levels were expected in red muscle because anadromous salmon do not feed during their freshwater spawning migration (Kadri et al. 1995) and must rely on stored reserves to fuel red muscle activity. White muscle is generally not used for aerobic swimming in fish and because of its poor vascularization is unlikely to mobilize FFAs required for sustained activity during spawning migration (Mommsen et al. 1980; Hochachka 1994). Lipid levels in the liver were much less reduced, likely because of the central role that this organ plays in lipid metabolism (Stryer 1988). Fatty acids are synthesized by the liver, esterified, secreted into the blood, and then transported to essential organs like muscle and brain. Thus, maintenance of lipid levels in the liver is essential, and lipid is believed to be mobilized from areas of less importance (e.g., pyloric caeca) and transferred to the liver during spawning migration (Jonsson et al. 1997).

Changes in $\delta^{13}C$ generally resembled those seen in lipid content during the spawning migration. Red muscle showed the greatest ^{13}C enrichment (about 4–5‰), and this effect was highly correlated with lipid levels in both sexes (Table 2; Fig. 5*a*). Of the three tissues, only red muscle showed continued ^{13}C enrichment over the entire study period (Fig. 3*a*). Liver and white muscle $\delta^{13}C$ also became significantly more enriched, but as seen in lipid content, changes were restricted to upstream migrants. Lipid is isotopically more ^{13}C depleted than other tissue components (DeNiro and Epstein 1977), and the lipid content of different tissues has been correlated with their $\delta^{13}C$ values (Tieszen et al. 1983). As tissues such as red muscle deplete lipid stores, either through mobilization of FFAs to other tissues or through catabolism of FFAs to sustain swimming activity (Keissling and Keissling 1993), $\delta^{13}C$ values should become more enriched. Our results are congruent with this hypothesis. Red muscle lost the greatest amount of lipid and showed the greatest enrichment in $\delta^{13}C$. Liver tissue lost less lipid than red muscle but more than white muscle and, as expected, showed intermediate $\delta^{13}C$ values.

Differences between $\delta^{13}C$ values in lipid-extracted and nonextracted red muscle tissue suggested that lipid reserves may not have been completely expended by the kelt stage, even though salmon had migrated, spawned, and spent one full year in freshwater without feeding. After samples were originally analyzed for lipid and protein concentrations and

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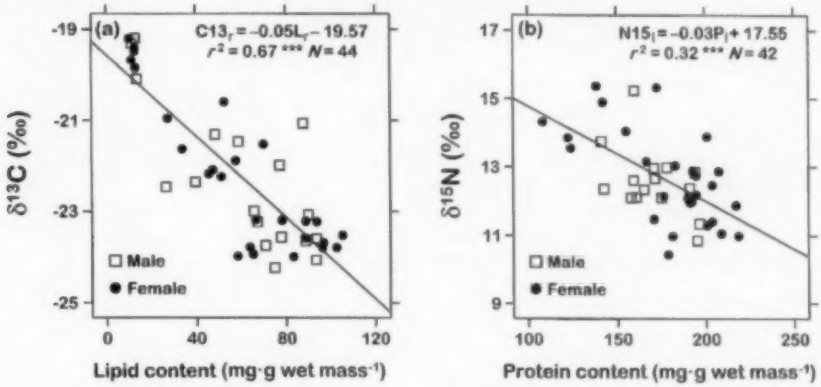
Fig. 5. Correlation between (*a*) lipid content (L_r) and $\delta^{13}C$ ($C13_r$) in red muscle and (*b*) protein content (P_l) and $\delta^{15}N$ ($N15_l$) in liver tissue in adult Atlantic salmon collected from the Exploits River, Newfoundland, in 1996 during their spawning migration. Model 2 regression equations, coefficients of determination (r^2), sample size (N), and significance levels (***$P < 0.001$) are also given.



$\delta^{13}C$ and $\delta^{15}N$ values, a common extraction method was used to remove all lipids from red muscle, and the tissue was reanalyzed for $\delta^{13}C$. Surprisingly, nonextracted samples from kelts (site 7) were still > 1‰ more ^{13}C depleted than extracted samples (Fig. 4), indicating that lipid levels were not completely exhausted. Booth (1998) suggested that because plasma nonesterified fatty acids were still at levels that could support locomotion and metabolism in kelts, other fuels may be important to adult Atlantic salmon during their overwintering stage in freshwater. Other available energy sources include carbohydrate and protein. Of the two, protein is the most likely to be used under nutritionally stressed conditions because salmonids possess a metabolic pathway (i.e., gluconeogenesis) that can convert amino acids into glucose and because carbohydrate stores are very small (Higgs et al. 1995).

Protein content and $\delta^{15}N$

Significant protein losses occurred in Atlantic salmon over the migration period (Fig. 2*b*). Reductions were greatest in liver and white muscle, where about 30% of the protein pool was metabolized during freshwater residency, and mostly during the overwintering kelt stage. These losses were similar to those reported by Mathisen et al. (1988) for anadromous sockeye salmon in Iliamna Lake, Alaska. Despite protein losses in all tissues, only liver showed consistent enrichment in $\delta^{15}N$ (Fig. 3*b*). This was surprising because we had expected protein catabolism to cause enrichment in the ^{15}N pool of all tissues.

Metabolic reorganization may be responsible for the different isotopic responses in these tissues. In order for $\delta^{15}N$ values to become more positive, a tissue must (*i*) be provided with substrates for anabolism that are isotopically more enriched than the initial N pool and (or) (*ii*) preferentially use (or remove) isotopically depleted substrates during catabolism (or mobilization). There is unlikely to be a preference for either isotopically light or isotopically heavy substrates during catabolism or removal; thus, nutrient losses under these conditions should occur with little or no change in stable-isotope ratios (Galimov 1985). However, ^{15}N enrichment does occur through protein recycling processes, such as transamination and deamination (Gaebler et al. 1966), and anabolic precursors can have higher $\delta^{15}N$ values than the original tissue pool. Because the liver in salmonids is the site of fatty acid metabolism, gluconeogenesis, and (in females) vitellogenin production (Tyler et al. 1988), tissue turnover and recycling are plausible explanations for the observed enrichment in liver $\delta^{15}N$. During the spawning migration, protein was removed principally from white muscle (Mommsen et al. 1980), and possibly red muscle, without isotopic fractionation. Amino acids (i.e., precursors for protein anabolism) were then obtained through recycling processes and their isotopically enriched forms transferred to liver cells, where minimal protein levels were required to sustain proper metabolic function. Protein reorganization has been observed previously in maturing female Atlantic salmon (Martin et al. 1993), and the significant correlation between protein content and $\delta^{15}N$ in female salmon in our study supports this argument (Table 2; Fig. 5*b*).

Lack of enrichment in muscle $\delta^{15}N$ in starved fish is contrary to what has been reported for nutritionally stressed birds (Hobson et al. 1993). These authors found significant enrichment (about 1.5‰) in pectoral muscle $\delta^{15}N$ in wild Ross' geese. It seems quite surprising that, despite the tremendous costs associated with the spawning migration, Atlantic salmon showed no consistent ^{15}N enrichment in either red or white muscle. This may highlight an interesting difference attributable to the specific metabolic demands placed on poikilotherms and homeotherms during periods of nutritional stress, and we feel that this subject requires further investigation.

Failure to see a trend in muscle $\delta^{15}N$ in Atlantic salmon may also be a result of sampling this particular population. Newfoundland stocks, including those of the Exploits River, have a high proportion of grilse, or one-sea-winter adult salmon (Power 1981). Jonsson et al. (1997) suggested that smaller one-sea-winter salmon use proportionally less of their energy reserves for spawning than larger multi-sea-winter fish. Thus, Atlantic salmon stocks with a high proportion of multi-sea-winter fish should reflect greater effects of

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nutritional stress in their muscle $\delta^{15}N$ values than the salmon sampled in our study, which were mostly (> 90%) one-sea-winter fish.

Another explanation for the lack of ^{15}N enrichment in muscle may be that the spawning migration of Atlantic salmon up the Exploits River is relatively easy. Jonsson et al. (1991) suggested that most of the metabolic costs associated with the spawning migration in Atlantic salmon can be met by lipid reserves. These authors found that Atlantic salmon utilized about 51% of their stored energy content during the spawning migration, whereas semelparous Pacific salmonids, such as sockeye salmon, used almost 80% of their total energy reserves (Brett 1986). In this case, differences in metabolic demand may be related to the length of freshwater migration. For example, the total distance traveled by Atlantic salmon on the Exploits River was only 112 km. Pacific salmon in the Fraser River, British Columbia, can travel distances of > 800 km. Therefore, effects of nutritional stress on muscle $\delta^{15}N$ may be more likely in Pacific salmonids. Unfortunately, preliminary work by Kline et al. (1993) showed only small, nonsignificant differences between muscle $\delta^{15}N$ of fresh and spawned-out sockeye salmon. However, further research in this area may still be warranted, as prespawning adult salmon, which spend in excess of 1 year in freshwater without feeding, may experience metabolic costs that are drastic enough to cause enrichment in muscle $\delta^{15}N$. Atlantic salmon in the Ungava region of northern Labrador are likely candidates for this type of prolonged ^{15}N enrichment (Power 1981).

Implications for other studies

Our results suggest that a range of at least 4–6‰ can be expected in both the $\delta^{13}C$ and $\delta^{15}N$ values of anadromous salmonids during their freshwater spawning migration (Fig. 5) and that this variability is attributable to changes in tissue concentrations of both protein and lipid. Failure to recognize the importance of this "nutritional effect" could lead to inaccurate conclusions when inferring food web relationships from stable-isotope ratios. Firstly, migrating anadromous salmon with a $\delta^{13}C$ of about –25‰ may be erroneously interpreted as having come from a different feeding location than fish with a $\delta^{13}C$ of about –20‰, or they may be mistakenly thought to have fed while in freshwater (because riverine sources usually mimic terrestrial $\delta^{13}C$ signatures near about –28‰, Peterson and Fry 1987). As well, anadromous salmonids with contrasting $\delta^{15}N$ values (e.g., 11 and 15‰) might be misconstrued as having fed at different trophic levels. Pretreating samples via lipid extraction might be one way of reducing variability in $\delta^{13}C$, and this procedure has been advocated previously (Hobson and Clark 1992). However, lipid extraction does not deal with excessive variability in $\delta^{15}N$ and in fact may have adverse effects on tissue $\delta^{15}N$ (R.R. Doucett, unpublished data).

Secondly, inaccuracies might arise in stable-isotope studies when nutrients derived from migrating salmon are incorporated into freshwater and (or) terrestrial food webs (e.g., Kline et al. 1990, 1993; Bilby et al. 1996; Hildebrand et al. 1996; Ben-David et al. 1997, 1998). Our results suggest that if studies incorporate $\delta^{13}C$ and $\delta^{15}N$ from fresh fish, when values representative of spawned or dying fish would be more suitable (or vice versa), then all further interpretation may be flawed. It is important to recognize the shift in stable-isotope ratios of migrating fish so that they may be sampled at the most appropriate time and place for valid comparison.

Finally, concern might also be warranted when liver $\delta^{15}N$ is used (as a proxy for trophic position) in stable-isotope/biomagnification studies involving migrating fishes (e.g., Kidd et al. 1995). Our results showed that liver $\delta^{15}N$ is enriched during spawning migration and that upstream migrants had lower liver $\delta^{15}N$ values than overwintering kelts. Because $\delta^{15}N$ is considered to be a good correlate of trophic position (Minagawa and Wada 1984), upstream migrants and overwintering kelts might have been erroneously interpreted as existing at different trophic levels. Such errors would lead to misinterpreting the biomagnification potential of different chemicals in these fish. Use of red or white muscle (or possibly whole bodies) instead of liver tissue may circumvent the nutritional effect seen in liver $\delta^{15}N$, as we did not find any significant ^{15}N enrichment in muscle tissue in our study.

Conclusions

Changes in the stable-isotope ratios of Atlantic salmon can result from losses in energy reserves as adults migrate upstream, spawn, and overwinter in freshwater without feeding. Isotopic enrichment in $\delta^{13}C$ results from the loss of lipid, whereas enrichment in $\delta^{15}N$ relates to protein turnover and recycling. The greatest effects were observed in red muscle and liver tissue, where changes in lipid and protein content were strongly correlated with $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Lipid losses and subsequent enrichment in $\delta^{13}C$ were most apparent during upstream migration. Protein losses occurred only during overwintering, as a result of the need for supplemental energy during the latter stages of prolonged fasting. These findings suggest that caution should be exercised when inferring food web relationships from stable-isotope data taken from spawning anadromous salmonids or any fish species undergoing extensive migration or any other excessive metabolic cost during a period of nutritional stress. Further laboratory research, combining stable-isotope techniques with nutritional information gathered from physiological studies (see Gannes et al. 1997), would likely improve the interpretation of stable-isotope ratios in field-collected data.

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Effects of preservatives and acidification on the stable isotope ratios ($^{15}N:^{14}N$, $^{13}C:^{12}C$) of two species of marine animals

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Abstract: When animal tissues are prepared for stable isotope ratio analysis, they may or may not be treated with acid prior to analysis to remove carbonates and are loaded into tin or silver weigh boats for quantitative combustion. The effects of these methodological variations are poorly known. The effects of various preservation methods on isotopic compositions are also poorly known. We tested the effects of four preservation methods, (*i*) formalin, (*ii*) formalin followed by a transfer to ethanol (formalin/EtOH), (*iii*) saturated mercuric chloride solution, and (*iv*) freezing/freeze-drying, on the carbon and nitrogen isotopic composition of the muscle tissue of juvenile winter flounder (*Pleuronectes americanus*) and the tails (including exoskeleton) of mud shrimp (*Crangon septemspinosa*). Freezing and freeze-drying were the only preservation methods that did not affect stable isotope ratios of carbon and nitrogen. Formalin, formalin/EtOH, and saturated mercuric chloride solution produced significant increases in $\delta^{15}N$ values (0.5–1.4‰) and decreases in $\delta^{13}C$ values (0.6–2.3‰) compared with frozen samples. There was also an increase in the variability of $\delta^{15}N$ and (or) $\delta^{13}C$ values. We also tested the effects of acidification by comparing samples that were acidified either by fuming with concentrated HCl or by the direct application of 1 N HCl containing 1.0% platinum chloride (a combustion catalyst) to unacidified samples. Neither concentrated HCl fumes nor HCl/platinum chloride had a significant effect on the $\delta^{15}N$ or $\delta^{13}C$ values of either species compared with unacidified samples. Therefore, acidification may be unnecessary in the preparation of some marine animals. Finally, we compared the effects of two types of sample boats: tin and silver. We found no significant effect of boat material on the $\delta^{15}N$ or $\delta^{13}C$ values of either species.

Résumé : Quand les tissus d'animaux sont préparés pour analyse des ratios d'isotopes stables, ils peuvent ou non être traités à l'acide avant analyse pour qu'en soient retirés les carbonates, et sont placés dans des nacelles de fer-blanc ou d'argent en vue de la combustion quantitative. Les effets de ces variations méthodologiques sont peu connus. Les effets des diverses méthodes de préservation sur les compositions isotopiques sont aussi mal connus. Nous avons testé les effets de quatre méthodes de préservation, (*i*) formol, (*ii*) bain de formol suivi d'un transfert dans l'éthanol, (*iii*) solution saturée de chlorure mercurique et (*iv*) congélation/lyophilisation, sur la composition isotopique en carbone et en azote du tissu musculaire de plies rouges (*Pleuronectes americanus*) juvéniles et les queues (incluant l'exosquelette) de crevette grise de sable (*Crangon septemspinosa*). La congélation et la lyophilisation étaient les seules méthodes de préservation qui n'ont pas influé sur les ratios des isotopes stables de carbone et d'azote. Le formol, le bain de formol suivi d'un transfert dans l'éthanol et la solution saturée de chlorure mercurique ont entraîné une hausse significative des valeurs de $\delta^{15}N$ (0,5–1,4‰) et une baisse significative des valeurs de $\delta^{13}C$ (0,6–2,3‰) comparativement aux échantillons congelés. Nous avons aussi observé un accroissement de la variabilité des valeurs de $\delta^{15}N$ et (ou) $\delta^{13}C$. Nous avons aussi testé les effets de l'acidification en comparant des échantillons qui ont été acidifiés par exposition aux vapeurs de HCl concentré ou par application directe de HCl 1 N renfermant 1,0% de chlorure de platine (un catalyseur de combustion) à des échantillons non acidifiés. Ni les vapeurs de HCl concentré ni le HCl/chlorure de platine n'ont eu un effet significatif sur les valeurs de $\delta^{15}N$ ou $\delta^{13}C$ des deux espèces comparativement aux échantillons non acidifiés. Ainsi, l'acidification pourrait être inutile dans la préparation de certains animaux marins. Enfin, nous avons comparé les effets de deux types de nacelles pour échantillons : les nacelles en fer-blanc et les nacelles en argent. Nous avons observé que la matière dont sont faites les nacelles n'a pas d'effet significatif sur les valeurs de $\delta^{15}N$ ou $\delta^{13}C$ des deux espèces.

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Introduction

Stable isotopic studies might often be enhanced with archived samples from preexisting collections. However, there has been uncertainty as to the effects of preservatives on stable isotope ratios. For example, the presence of carbon or nitrogen in chemical preservatives might be expected to contaminate preserved specimens with respect to $\delta^{13}C$ or $\delta^{15}N$ analyses. Samples used in stable isotopic studies of food webs (e.g., Hesslein et al. 1991; Sholto-Douglas et al. 1991; Rau et al. 1992; Wainright et al. 1993; Welch and Parsons 1993; Wainright and Fry 1994) are typically preserved by either drying or freezing them immediately or preserving them with saturated mercuric chloride ($HgCl_2$) solution, but little is known about the effects of preservatives on stable isotope ratios of carbon and nitrogen.

Because organic samples often contain carbonates that could potentially alter $\delta^{13}C$ values, it is common for samples to be acidified before analysis (Jackson et al. 1986; Hobson and Welch 1992). When samples are prepared for continuous-flow isotope ratio mass spectrometry, they are typically loaded into tin sample weigh boats for combustion. It may be desirable to acidify the samples directly in the sample boats. However, because tin boats degrade rapidly in the presence of acid, silver boats (which are more acid resistent) are sometimes used instead. It is not known what effects the boat material has on stable isotope ratios. There is the potential for incomplete combustion in silver boats, which do not undergo flash combustion (Carlo Erba Instruments 1990).

The present study was undertaken to determine the effects of several commonly used preservatives on the carbon and nitrogen stable isotope ratios of juvenile winter flounder (*Pleuronectes americanus*) and mud shrimp (*Crangon septemspinosa*). The effects of acidification, using either concentrated HCl fumes or the direct application of dilute liquid acid, were also evaluated. Samples were combusted in both silver and tin sample boats to see what effect, if any, boat type would have on stable isotope ratios of carbon and nitrogen.

Materials and methods

The effects of four preservation methods were tested: (*i*) 10% formalin, (*ii*) 10% formalin followed by a transfer to 90% ethanol (hereafter referred to as formalin/EtOH), (*iii*) seawater containing a saturating concentration of $HgCl_2$ (solubility about 690 $mg \cdot L^{-1}$), and (*iv*) freezing at –80°C. We also tested the effects of acidification by comparing samples that were acidified either by fuming with concentrated HCl or by the direct application of 1 N HCl containing 1.0% platinum chloride (a combustion catalyst, hereafter referred to as HCl/$PtCl_2$). Finally, we compared the effects of two types of sample boats: tin, which undergoes flash combustion in the presence of oxygen, and silver, which does not flash combust. Two species were used in these comparisons: *C. septemspinosa* and juvenile *P. americanus*. *Crangon septemspinosa* was chosen to represent animals with an exoskeleton containing $CaCO_3$ (which was left intact to increase the likelihood of detecting acidification effects), whereas *P. americanus* was chosen to minimize the contribution of inorganic carbon to $\delta^{13}C$ values.

Preservative and acid effects

Crangon septemspinosa and *P. americanus* were collected from a cove along the southern end of Long Beach Island, New Jersey, in Little Egg Harbor, using a 1-m beam trawl on 17 July 1995. Groups of five individuals of *P. americanus* and *C. septemspinosa* were randomly assigned to the four preservative treatments.

For the formalin/EtOH treatments, the transfer from formalin to ethanol was performed about 2 months after the initial preservation in formalin. After another 2 months, the samples were prepared for analysis.

A piece of muscle tissue from three *P. americanus* in each treatment was removed from below the dorsal fin and just above the midline, posterior to the body cavity. Skin was removed and the muscle samples dried (55°C for 24 h or until completely dried) in precombusted (500°C for 5 h) glass vials. Three *C. septemspinosa* from each treatment were selected, the tails removed, the digestive tracts removed from the tails (where possible), and the tails dried in a manner similar to that for fish muscle. Dried samples were ground to a fine powder with a Wig-L-Bug™ (Crescent Dental Corp.) and stored in glass vials in a desiccator.

For $\delta^{13}C$ analysis, 0.343–0.405 mg of the homogeneous powder was loaded into silver boats and then placed into a closed container in the presence of concentrated HCl fumes for 24 h. Subsamples for $\delta^{15}N$ analysis (0.485–0.606 mg) were treated similarly but were not acidified and were loaded into tin boats. Loaded sample boats were stored in a desiccator until analysis.

The two preservative treatments that we predicted would have the most different stable isotope ratios were freezing and formalin/EtOH. Therefore, two of the three *P. americanus* and *C. septemspinosa* from these treatments were chosen at random to prepare two additional treatments so that the effects of acidification and preservation might be separated. Because samples analyzed for $\delta^{15}N$ were normally not acidified, the two additional treatments to test for acidification effects on $\delta^{15}N$ values were to acidify them with fuming HCl (as described above) and with HCl/$PtCl_2$. Acidification with HCl/$PtCl_2$ was done by applying enough liquid acid directly onto the samples in silver boats to completely moisten the samples and then allowing them to dry in a dessicator. Because samples analyzed for $\delta^{13}C$ were normally acidified with fuming HCl, the two additional treatments to test acidification effects on $\delta^{13}C$ values were to analyze them unacidified and after acidification with HCl/$PtCl_2$.

Samples for this portion of the study were analyzed via continuous flow using a Carlo Erba elemental analyzer coupled with a VG Prism stable isotope mass spectrometer.

Effects of boat type and freezing

Additional treatments were prepared to compare samples dried fresh with samples that had first been frozen or freeze-dried and to compare samples that were analyzed in tin boats with those analyzed in silver boats. *Crangon septemspinosa* were collected from Little Sheepshead Creek in the Mullica River estuary in Tuckerton, N.J., on 24 April 1996. Four live *C. septemspinosa* were selected at random, the tails separated from the body, and, where possible, the digestive tracts removed. The tails were then divided longitudinally into thirds. One third was chosen randomly, placed in a precombusted (500°C for 5 h) glass vial, and dried at 55°C. The other two thirds were frozen at –80°C. After several days, one of the two frozen thirds was chosen randomly and dried at 55°C in a precombusted glass vial and the other was freeze-dried. Dried samples were ground to a fine powder, as described above. Each sample was prepared for analysis in three different ways: in tin boats unacidified, in tin boats after exposure to fuming HCl, and in silver boats followed by exposure to fuming HCl.

The samples for this portion of the study were analyzed via continuous flow on a Europa Scientific ANCA-SL preparatory unit coupled to a Europa Scientific 20/20 stable isotope mass spectrometer.

Statistical comparisons were made with the aid of a statistical software package (SAS Institute, Inc. 1995). Standard ANOVA

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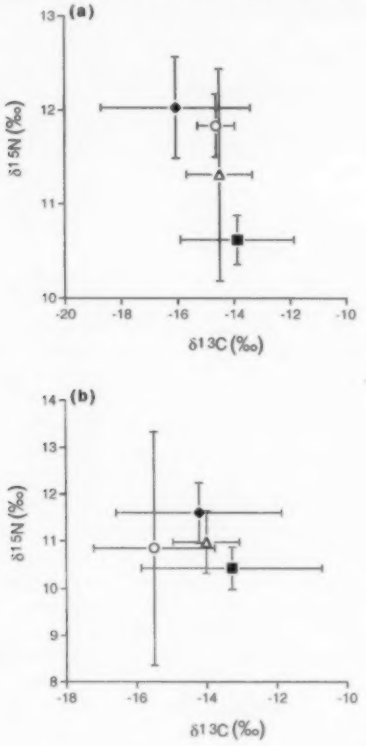
Fig. 1. Mean $\delta^{15}N$ and $\delta^{13}C$ values (±95% confidence intervals) of (*a*) *P. americanus* and (*b*) *C. septemspinosa* (from 1995 collection) obtained using four different preservation methods (n = 3 each). Squares represent freezing; diamonds represent formalin/EtOH; circles represent formalin; and triangles represent $HgCl_2$.



procedures (Proc GLM) were used to detect treatment effects. Follow-up comparisons were made with an SNK post hoc test.

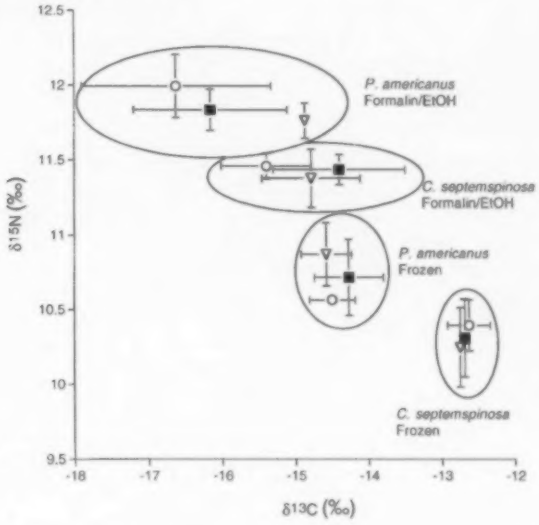
Results

Preservative and acid effects

There was a significant treatment effect of preservatives on both nitrogen ($p < 0.01$) and carbon ($p < 0.05$) stable isotopes of *P. americanus* muscle tissue (Fig. 1*a*). Follow-up tests revealed that the formalin/EtOH treatment exhibited elevated $\delta^{15}N$ when compared with frozen samples (mean $\delta^{15}N$ ± SD = 12.03 ± 0.22 and 10.62 ± 0.10‰, respectively, n = 3 each, $p < 0.01$). However, the $\delta^{13}C$ values were significantly lower than those of frozen samples (–16.06 ± 1.07 and –13.89 ± 0.81‰, respectively, n = 3 each, $p < 0.01$).

The overall effect of preservatives on nitrogen ($p > 0.1$) and carbon ($p > 0.05$) stable isotopes of *C. septemspinosa* was not significant (Fig. 1*b*). However, when the formalin/EtOH and frozen *C. septemspinosa* treatments were compared to test both the effects of the acidification methods and the potential interaction between preserving and acidifying (Fig. 2), both the mean $\delta^{13}C$ and $\delta^{15}N$ values (for all of the acidification methods combined) of the formalin/EtOH treatment were significantly different from those of the frozen treatment. The difference was >2‰ for $\delta^{13}C$ values (–14.84 ± 0.93 and –12.69 ± 0.20‰, respectively, n = 6 each, $p < 0.01$) and >1‰ for $\delta^{15}N$ values (11.42 ± 0.16 and 10.32 ± 0.27‰, respectively, n = 6 each, $p < 0.01$).

Fig. 2. Mean $\delta^{15}N$ and $\delta^{13}C$ values (±1 SE) for the treatments of *P. americanus* and *C. septemspinosa* (from 1995 collection) that were unacidified (circles, n = 2 for both species), acidified with concentrated HCl fumes (squares, n = 2 for both species), or acidified with liquid 1 N HCl containing 1.0% $PtCl_2$ (triangles, n = 2 for both species except n = 1 for *P. americanus* formalin/EtOH $\delta^{13}C$).



Samples preserved with formalin only had stable isotope ratios similar to those preserved with formalin/EtOH. Formalin-preserved samples of *P. americanus* exhibited significantly enriched $\delta^{15}N$ compared with frozen samples (11.83 ± 0.14 and 10.62 ± 0.10‰, respectively, n = 3 each, $p < 0.01$), but the $\delta^{13}C$ values were not significantly different (–14.63 ± 0.27 and –13.89 ± 0.81‰, respectively, n = 3 each, $p > 0.2$). Formalin-preserved samples of *C. septemspinosa* exhibited greater variability in $\delta^{15}N$ values compared with frozen ones (95% confidence intervals = ± 2.49 and ±0.45, respectively).

$HgCl_2$ also had an isotopic effect similar to the formalin and formalin/EtOH treatments. Samples of *P. americanus* preserved in $HgCl_2$ had significantly greater $\delta^{15}N$ values compared with frozen samples (11.31 ± 0.45 and 10.62 ± 0.10‰, respectively, n = 3 each, $p < 0.02$), and the results were more variable (95% confidence intervals = ±1.13 and ±0.26, respectively). The effect on carbon isotopes was also similar to those of the other two preservatives. $HgCl_2$-preserved samples of *P. americanus* had $\delta^{13}C$ values that were lower relative to frozen samples, but the difference was not significant (–14.51 ± 0.27 and –13.89 ± 0.81‰, respectively, n = 3 each, $p > 0.3$).

Acidification with either concentrated HCl fumes or HCl/$PtCl_2$ did not significantly effect the $\delta^{15}N$ or $\delta^{13}C$ values of *P. americanus* or *C. septemspinosa* (Fig. 2).

Effects of boat type and freezing

Fresh-dried subsamples of *C. septemspinosa* did not differ isotopically from subsamples that had been either frozen or

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freeze-dried prior to analysis ($p > 0.5$ for both carbon and nitrogen). The difference in $\delta^{13}C$ values was <0.3‰, and the difference in $\delta^{15}N$ values was <0.2‰.

There were no significant differences in either the $\delta^{15}N$ or $\delta^{13}C$ values of *C. septemspinosa* that were analyzed in silver boats versus tin boats ($p > 0.5$ for both carbon and nitrogen). The difference in $\delta^{13}C$ values was <0.3‰, and the difference in $\delta^{15}N$ values was <0.1‰. There also were no significant differences in percent carbon and nitrogen composition between these treatments ($p > 0.5$).

Discussion

Preservatives clearly affected the stable isotopic compositions of *P. americanus* and *C. septemspinosa*. Although the effects of formalin and formalin/EtOH were not unexpected, the effect of $HgCl_2$ was surprising because it does not contain either carbon or nitrogen. An enrichment of ^{15}N and depletion of ^{13}C occurred in all of the preserved treatments and suggests that preservatives may prevent the loss of compounds enriched in ^{15}N and ^{12}C. Alternatively, preservatives may promote the leaching of compounds that are enriched in ^{14}N and ^{13}C. The data do not allow us to distinguish between these effects. Preserved tissues may also have different combustion characteristics than unpreserved tissues.

In our study, the variability of $\delta^{15}N$ values for both species was increased by the use of preservatives, while the $\delta^{13}C$ values of the frozen treatments had greater variability than some of the preserved treatments (Fig. 1). It is likely that a larger sample size would be necessary to determine the effect of preservatives on the variability of these measurements.

Acidification did not affect the $\delta^{13}C$ or $\delta^{15}N$ values of the species in this study. In a similar investigation, Bunn et al. (1995) found that acidification had no significant effect on $\delta^{13}C$ values of two kinds of samples, penaeid shrimp (*Metapenaeus moyebi*) and seagrass (*Enhalus acoroides*). Our results agree, in part, with the findings of Bunn et al. (1995) in that acidification had no significant effect on $\delta^{13}C$ values of invertebrates that have an exoskeleton, even when the exoskeleton was not removed (in our case). However, Bunn et al. (1995) reported that acidification with 0.1 N HCl followed by rinsing with distilled water caused a significant enrichment of $\delta^{15}N$ values of *M. moyebi*. They also determined that the *E. acoroides*, treated similarly but with 1 N HCl, had $\delta^{15}N$ values that were significantly lower (depleted) and more variable than *E. acoroides* that was not acid rinsed. In the present study, acidification had no significant effect on the $\delta^{15}N$ values of *P. americanus* or *C. septemspinosa*. The difference in acidification effects seen in these two studies may stem from the fact that the acidification procedures of Bunn et al. (1995) included a rinsing step. Rinsing may remove dissolved compounds leached from the samples by acid. Our results suggest that acidification may be an unnecessary preparation step for at least some types of animal tissue samples, including some that have exoskeletons.

Although we found no measureable effect of freeze-drying on carbon stable isotope ratios of the relatively large animal tissue samples analyzed here (about 50 µmol of carbon), Fry et al. (1993) found that freeze-drying may significantly affect the $\delta^{13}C$ values of certain samples of low organic content (about <3 µmol of carbon) because of large blanks.

Silver sample boats do not undergo flash combustion. A concern about their use in mass spectrometry is that they may not allow samples to undergo complete combustion. However, in this study, samples combusted in silver boats showed no significant differences in carbon or nitrogen stable isotopic compositions or the relative percentages of either element when compared with samples combusted in tin boats (which do flash combust). These findings suggest that combustion was comparably efficient with both types of sample boats. However, because this comparison was based on a single sample type, further investigations with other kinds of samples may be warranted.

The results presented here have considerable implications for studies involving stable isotopes. The differences between the $\delta^{15}N$ values of preserved samples of *P. americanus* and *C. septemspinosa* and unpreserved samples ranged from 0.5 to 1.4‰ for both species combined. If one assumes that there is a 3–4‰ difference in $\delta^{15}N$ values between consumer and prey (DeNiro and Epstein 1981; Minigawa and Wada 1984; Fry 1988), then the use of preserved samples might lead one to overestimate the trophic level of those organisms. Carbon stable isotope ratios, too, are sometimes used as an indicator of trophic level (McConnaughey and McRoy 1979; Rau et al. 1983; Mills et al. 1984); a trophic level enhancement of 1–2‰ is often assumed. Thus, preservatives may lead to a substantial underestimation of trophic level based on carbon stable isotopes. For example, the $\delta^{13}C$ values of *C. septemspinosa* preserved with formalin were more than 2‰ (about one or two trophic levels) lower than frozen samples (see Fig. 1*b*). Apart from potential effects on estimates of trophic level, preservative effects may be important in their influence on isotopic results in general, depending on the magnitude of the isotopic signal relative to background variation.

This study represents the first attempt to examine the effects of preservatives on the stable isotope ratios of two species of aquatic animals. Further tests should be done to evaluate whether these findings can be generalized to other kinds of preservatives and sample types. Until then, freezing should be considered the only method of preserving samples that will not affect stable isotope ratios.

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Stable nitrogen and carbon isotopic composition of seston and sediment in two Adirondack lakes

J.S. Owen, M.J. Mitchell, and R.H. Michener

Abstract: Values of $\delta^{15}N$ and $\delta^{13}C$ natural abundance in seston were compared in two Adirondack lakes that differed with respect to water column NO_3^- concentration. Seston $\delta^{15}N$ values in Arbutus Lake averaged 2.5 ± 0.18‰ (mean ± SE) and 2.7 ± 0.15‰ at 2 and 6 m depth, respectively. NO_3^- concentrations in Arbutus Lake were generally low (range 0–13 μM) with greatly decreased concentrations during the summer; temporal changes in seston $\delta^{15}N$ values and water column NO_3^- concentrations were inversely correlated. NO_3^- concentrations in Dart's Lake were higher (range 12.9–22.4 μM) and the magnitude of the decreased summer NO_3^- concentration lower. Seston $\delta^{15}N$ values in Dart's Lake averaged 1.3 ± 0.12 and 1.1 ± 0.07‰ at depths of 6 and 14 m, respectively. There was no significant correlation between seston $\delta^{15}N$ values and water column NO_3^- concentration in Dart's Lake. Average seston $\delta^{13}C$ values in Arbutus Lake and Dart's Lake were –29.8 and –28.7‰, respectively. More information about the distribution of stable isotopes for a wide variety of freshwater systems is needed to increase our understanding of the patterns observed in this study.

Résumé : Les valeurs naturelles du $\delta^{15}N$ et $\delta^{13}C$ du seston de deux lacs des Adirondacks qui différaient de par la concentration de NO_3^- de la colonne d'eau ont été comparées. Les $\delta^{15}N$ moyens du lac Arbutus étaient, respectivement, de 2,5 ± 0,18‰ (moyenne ± ET) et de 2,7 ± 0,15‰ à 2 et 6 m de profondeur. Les concentrations de NO_3^- du lac Arbutus étaient généralement faibles (gamme de 0 à 13 μM) et diminuaient de façon importante au cours de l'été; les variations dans le temps du $\delta^{15}N$ du seston et des concentrations de NO_3^- de la colonne d'eau présentaient une corrélation inverse. Les concentrations de NO_3^- du lac Dart étaient supérieures (gamme de 12,9 à 22,4 μM) et l'amplitude du déclin estival du NO_3^- était inférieure. Le $\delta^{15}N$ du lac Dart s'élevait en moyenne à 1,3 ± 0,12‰ et à 1,1 ± 0,07‰ pour, respectivement, des profondeurs de 6 et 14 m. Il n'y avait pas de corrélation significative entre le $\delta^{15}N$ du seston et les concentrations de NO_3^- de la colonne d'eau pour le lac Dart. Les $\delta^{13}C$ moyens des lacs Arbutus et Dart étaient, respectivement, de –29,8‰ et de –28,7‰. Il faudra mieux connaître la répartition des isotopes stables dans une large gamme de systèmes dulcicoles afin d'être en mesure de mieux interpréter les tendances dégagées suite à la présente étude.

[Traduit par la Rédaction]

Introduction

Nitrogen has long been considered to be the element most limiting primary productivity in temperate forests (Vitousek and Howarth 1991). There is currently much interest in the possibility that N inputs to some regions are exceeding the plant and microbial demand of this nutrient, causing "N saturation" (Aber et al. 1989; Stoddard 1994). Aber et al. (1989) proposed that forest ecosystems will undergo a series of responses to chronic N additions. Indications of excess N inputs to forests that could occur in associated surface waters might include chronically elevated summer NO_3^- concentrations and increases in spring meltwater NO_3^- concentrations in surface waters (Stoddard 1994). One approach to document system-level responses to increased N inputs has been experimental N additions at the plot or watershed level (e.g., Kahl et al. 1993). Another approach, which can increase our understanding of various linkages between forests and surface waters by identifying nutrient sources and sinks, in addition to providing process-level information, uses naturally occurring stable isotope abundances.

Some research has suggested the possibility that forests that lose greater amounts of N have higher natural abundance ^{15}N values in vegetation (Högberg 1990). A survey of $\delta^{15}N$ values of foliage from the Walker Branch Watershed, Tennessee, indicated that foliar $\delta^{15}N$ was more positive in forest stands in relatively N-rich valleys than in those in more N-deficient upland sites (Garten 1993). Garten and Van Miegroet (1994) found a correlation between foliar ^{15}N enrichment and mineral soil N availability in this same watershed. Mechanisms for N loss from forests such as NH_3 volatilization, nitrification, and denitrification favor the removal

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Table 1. Lake and watershed characteristics for Arbutus and Dart's lakes.

	Arbutus Lake	Dart's Lake
Elevation (m)	513	536
Lake area (ha)	50	52
Watershed area (ha)	309	10 749
Volume (10^4 m^3)	139	381
Maximum depth (m)	8	17
Average depth (m)	3	7
Retention time (years)	0.6	0.05
pH[a]	6.49	5.25
NO_3^- (equiv.·L^{-1})[a]	7.0	26.0
Total P (mmol·L^{-1})	0.06–0.3	na
Chl *a* (mg·L^{-1})	na	0.2–2
Secchi disk depth (m)	3.4–5.0	3.5–5.7

Note: na, not available.

[a]Mean from June 1982 to April 1991 (from Driscoll and Van Dreason 1993).

of isotopically lighter ^{14}N (Shearer and Kohl 1986). The remaining N (substrate) will therefore become enriched in ^{15}N. If a general pattern of soil and foliar ^{15}N enrichment with increasing watershed N export occurs, then high N leaching sites should be characterized by loss of dissolved N species with more depleted $\delta^{15}N$ values compared with watersheds with lower N losses. Measurements of lake, stream, or soil solution NO_3^-, NO_4^+, and dissolved organic N isotopic composition, however, remain uncommon (Lajtha and Michener 1994).

Previous analysis of some Adirondack Long-Term Monitoring (ALTM) data, including our study sites, showed increasing NO_3^- concentration in nine of 15 drainage lakes from 1982 to 1991 (Driscoll and Van Dreason 1993). A spatial pattern of decreasing lake outlet NO_3^- concentration from west to east corresponding to higher N deposition in the western Adirondacks relative to the eastern and central regions has also been indicated (Driscoll et al. 1991).

We selected our study lakes to partially reflect this pattern. Dart's Lake is located in the southwestern Adirondacks, the area that was previously characterized as having elevated lake NO_3^- concentrations (Driscoll et al. 1991). Arbutus Lake is located in the central Adirondacks, an area containing several circumneutral lakes and lower NO_3^- concentrations (Baker et al. 1990). The objective of this study was to compare the stable N and C isotopic composition of seston and sediment in these two lakes, which differ in NO_3^- concentrations. Our main objective was to investigate the relationships between seston and sediment isotopic composition and water column NO_3^- concentrations.

Relatively few studies have investigated possible links between seston $\delta^{15}N$ natural abundance and water chemistry in freshwater systems. Previous studies, such as Kling et al. (1992), measured the natural abundance of C and N isotopes of particulate organic matter and zooplankton to document trophic relationships in several arctic lakes. Gu et al. (1996) analyzed the isotopic composition of surface sediments and plankton and suggested that $\delta^{15}N$ values of plankton in their study sites might have been related to the degree of N limitation. Bernasconi et al. (1997) found evidence for the influence of organic inputs from a water treatment plant on the isotopic composition of sedimenting material in Lake Lugano, Switzerland.

Methods

Study sites

Dart's Lake (43°48′N, 74°52′W) is located within the drainage basin of the North Branch of the Moose River in the southwestern Adirondacks. This watershed contains a series of lakes, the largest being Big Moose Lake, that drain into Dart's Lake. A subwatershed in the Big Moose Lake watershed, Pancake – Hall Creek, was the site of the soil and vegetation sampling. The dominant overstory vegetation species are mixed hardwoods and conifers including *Acer saccharum* (sugar maple), *Fagus grandifolia* (American beech), and *Betula alleghaniensis* (yellow birch) (Hurd et al. 1998).

Arbutus Lake (43°59′N, 74°15′W) is located in the central Adirondacks at the Huntington Forest. The soil and vegetation samples from this site were obtained 1 km south of the lake outlet. Dominant overstory trees at this site are *F. grandifolia*, *B. alleghaniensis*, and *Acer rubrum* (red maple) (Hurd et al. 1998). Selected lake and watershed characteristics for both systems are shown in Table 1.

Seston and sediment sampling

Cylindrical sediment traps (aspect ratio 10:1) of the same design used by David and Mitchell (1985) were installed near the deepest part of each lake and sampled during the ice-free season from September 1992 through October 1993. Traps were placed at 2 and 6 m depths in Arbutus Lake and at 6 and 14 m depths in Dart's Lake. Each trap contained three replicate cylinders and was sampled at 2- to 4-week intervals. A gravity corer was used to retrieve a sediment core adjacent to the sediment trap site. Cores were sectioned at 1-cm intervals, stored at 4°C, dried at 60°C, and pulverized prior to elemental analysis of C and N concentration and stable isotopic composition.

Water sampling

Water column samples were collected using a 1.2-L Kemmerer sampler during each seston collection. An additional sample for estimating suspended particulate material was collected from the depth corresponding to sediment trap position.

Soil and foliage collection

Foliage samples were obtained in 1993 from within plots without chemical treatment described in Mitchell et al. (1994). Live foliage of overstory *F. grandifolia* was sampled from the middle third of the tree crown with pole pruners. Leaf litter was collected by placing a polypropylene net under mature *A. saccharum* trees during leaf fall. Soil samples were collected near each tree after cutting away the overlying organic horizons and exposing the mineral soil. Soil and vegetation samples were pulverized and dried at 60°C prior to chemical and isotopic analysis.

Analysis

Dry mass of seston was determined by oven drying (60°C) the filtered contents of each trap (precombusted GFC filters, 500°C). A small correction for suspended particulate material was made by subtracting the mass of suspended particulate material in the water column on the day seston was collected. For selected samples, the filtered material from a single cylinder was gently scraped from the filter, placed in a clean vial, and dried at 60°C. These samples were pulverized and analyzed for C and N using a Perkin-Elmer 2400 elemental analyzer. C:N ratios are molar ratios throughout this paper.

Water samples were analyzed using methods described elsewhere (Driscoll and Van Dreason 1993). NO_3^- was analyzed by ion

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Fig. 1. Seston deposition in Arbutus and Dart's lakes during 1992 and 1993. ●, Arbutus Lake 2 m depth; ▼, Arbutus Lake 6 m depth; ○, Dart's Lake 6 m depth; ▽, Dart's Lake 14 m depth.

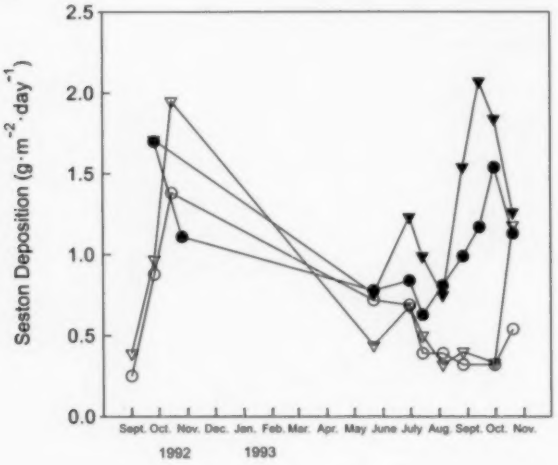
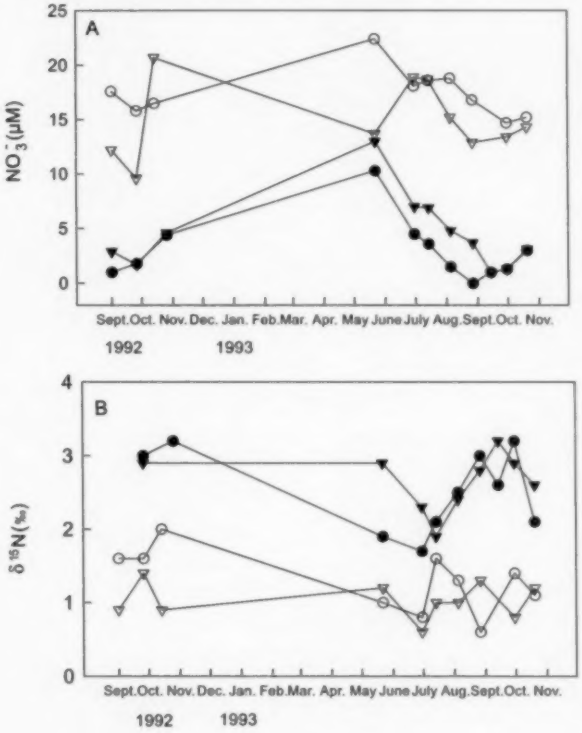


Fig. 2. (A) NO_3^- concentration and seston (B) $\delta^{15}N$ values in Arbutus and Dart's lakes during 1992 and 1993. ●, Arbutus Lake 2 m depth; ▼, Arbutus Lake 6 m depth; ○, Dart's Lake 6 m depth; ▽, Dart's Lake 14 m depth.



chromatography as soon after collection as possible (generally less than 48 h).

Stable isotopic C and N ratios of solid samples were determined on a Finnigan MAT Delta S mass spectrometer (Boston University Stable Isotope Laboratory). Filters containing seston were pulverized in a vial containing 1 g of CuO. This mixture was placed in a quartz tube (9 mm in diameter) and 0.5 g of Cu was added. The tubes were sealed and combusted in a programmable muffle furnace at 750°C (3 h), 650°C (2 h), and 600°C (1 h) and cooled overnight to room temperature.

All stable isotope data are presented using conventional per mill (‰) notation:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [R_{sample}/R_{standard} - 1] \times 1000$$

where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Stable isotope replicate analytical precision was usually within 0.2‰. Mean comparisons used either simple or paired t tests. Simple linear regression was used to analyze relationships between variables, but the nonparametric seasonal Kendall test (SKT) was used to estimate significance levels (Hirsch et al. 1982).

Results

Seston deposition was greater in Arbutus Lake ($p < 0.001$) (Fig. 1). In Arbutus Lake, seston deposition was greater at the lower trap depth than at the upper trap depth (1.4 ± 0.16 (9) and 1.1 ± 0.12 (9) g dry mass·m^{-2}·day^{-1} (mean ± SE (n)), $p < 0.05$). In Dart's Lake, seston deposition in upper and lower traps did not differ (0.6 ± 0.11 (10) and 0.7 ± 0.17 (10) g dry mass·m^{-2}·day^{-1}, respectively, $p > 0.05$). Dart's Lake showed higher NO_3^- concentrations than Arbutus Lake (Fig. 2), confirming results from earlier studies by Driscoll and Van Dreason (1993).

Temporal changes of seston $\delta^{15}N$ in Dart's and Arbutus lakes also showed different patterns (Fig. 2). Mean values of seston $\delta^{15}N$ differed between the lakes (2.6 and 1.1‰ for Arbutus Lake and Dart's Lake, respectively, $p < 0.001$) (Table 2). Seston $\delta^{15}N$ values in these Adirondack lakes were similar to that reported for particulate organic matter in several oligotrophic arctic lakes (Kling et al. 1992). A temporal trend of increasing $\delta^{15}N$ during the summer and early autumn was shown only for Arbutus Lake (Fig. 2). In Dart's Lake, epilimnetic seston $\delta^{15}N$ values were greater than values for seston collected at the greater depth ($p < 0.05$).

Seston $\delta^{13}C$ values also showed temporal changes, with $\delta^{13}C$ values generally decreasing during the midsummer period (Fig. 3). Mean seston $\delta^{13}C$ values in Arbutus Lake (–29.8‰) and Dart's Lake (–28.7‰) were similar to those found in 28 lakes in Quebec and Ontario (del Giorgio and France 1996). LaZerte (1983) reported somewhat more negative $\delta^{13}C$ values for phytoplankton in Lake Memphremagog, Quebec.

For these two oligotrophic lakes, seston $\delta^{13}C$ values varied between the two sites ($p < 0.01$) (Table 2). Even though these lakes are thermally stratified during the summer, we did not find a consistent difference in seston $\delta^{13}C$ values between upper and lower depths, as might be expected from recycling of respired CO_2 (LaZerte 1983). There were no differences in seston $\delta^{13}C$ values between the upper and lower depths for either lake ($p > 0.05$).

Total N concentration in seston did not differ between the sites ($p < 0.01$) (Table 3). Total C concentration in seston was higher in Dart's Lake ($p < 0.01$) (Table 3), resulting in differences in seston C:N ratios (Table 3). Seston C:N ratios (mean 11.7 and 15.2 for Arbutus Lake and Dart's Lake, re-

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Table 2. Seston $\delta^{15}N$ and $\delta^{13}C$ (mean ± SE (*n*)) in Arbutus and Dart's lakes during 1992 and 1993.

Site	Depth (m)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Arbutus Lake	2	2.5±0.18 (10)	–29.8±0.59 (8)
	6	2.7±0.15 (9)	–29.7±0.15 (8)
Dart's Lake	6	1.3±0.12 (10)	–28.8±0.12 (7)
	14	1.0±0.07 (10)	–28.6±0.27 (7)

Fig. 3. Seston $\delta^{13}C$ values in Arbutus and Dart's lakes during 1993. ●, Arbutus Lake 2 m depth; ▼, Arbutus Lake 6 m depth; ○, Dart's Lake 6 m depth; ▽, Dart's Lake 14 m depth.

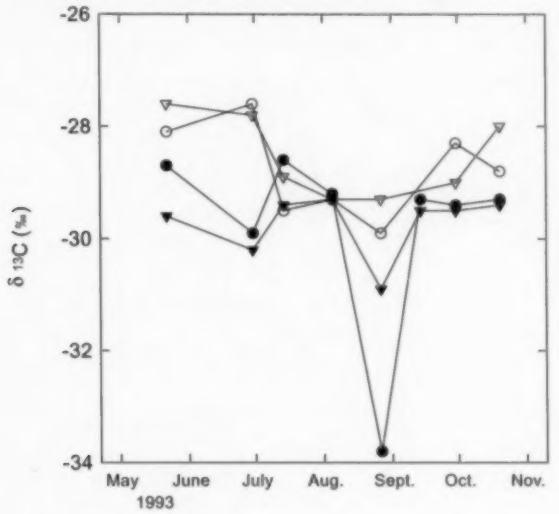

Table 3. Seston C and N concentrations (mmol·g^{-1}, mean ± SE (*n*)) and C:N ratios in Arbutus and Dart's lakes during 1993.

Site	Depth (m)		Concentration	C:N ratio
Arbutus Lake	2	C	16.8±0.84 (5)	11.9
		N	1.43±0.12 (5)	
	6	C	16.6±0.68 (5)	11.6
		N	1.46±0.15 (5)	
Dart's Lake	6	C	21.1±0.40 (2)	14.8
		N	1.43±0.01 (2)	
	14	C	21.5±1.74 (2)	15.6
		N	1.38±0.12 (2)	

Table 4. Isotopic composition (‰) and C:N ratios (minimum to maximum (*n*)) of sediment and some potential terrestrial sources of seston in Arbutus and Dart's lakes.

	Pancake – Hall Creek (Dart's Lake)	Huntington Forest (Arbutus Lake)
Fagus grandifolia foliage		
$\delta^{15}N$	–0.2 to 1.1 (3)	–1.0 to 0.4 (4)
$\delta^{13}C$	–31.3 to –30.6 (3)	–29.3 to –29.1 (2)
Acer saccharum litter		
$\delta^{15}N$	–1.5 to –0.6 (5)	–2.4 to –1.3 (5)
C:N ratio	62.7 to 72.7 (5)	52.8 to 61.7 (5)
B horizon soil		
$\delta^{15}N$	5.2 to 7.5 (5)	6.5 to 6.9 (5)
C:N ratio	22.3 to 25.1 (5)	22.3 to 28.6 (5)
Surface sediment (0–2 cm)		
$\delta^{15}N$	1.6	2.8
$\delta^{13}C$	–28.1	–29.7
C:N ratio	17.1	14.0

spectively) were lower than in potential seston sources from the watersheds (Table 4). In both Arbutus and Dart's lakes, upper and lower depth seston C and N concentrations did not differ ($p > 0.05$).

In forests near our study lakes, $\delta^{15}N$ values of *A. saccharum* leaf litter ranged from –2.4 to –1.3‰ at Huntington Forest and from –1.5 to –0.6‰ at Pancake – Hall Creek (Table 4). *Fagus grandifolia* foliage $\delta^{13}C$ at the Pancake – Hall Creek site (–30.9 ± 0.22‰ (3)) had lower $\delta^{13}C$ values ($p < 0.05$) than at Huntington Forest (–29.0 ± 0.21‰ (5)). Mineral soil (B horizon) $\delta^{15}N$ showed a greater range at the Pancake – Hall Creek site compared with Huntington Forest, but mean values were not significantly different ($p > 0.05$).

Sediment (0–2 cm) $\delta^{13}C$ values in Arbutus Lake were isotopically lighter than surface sediment values in Dart's Lake (–29.7 and –28.1‰, respectively). Surface sediment from Dart's Lake had a mean $\delta^{15}N$ value of 1.6‰ versus a value of 2.8‰ in Arbutus Lake (Table 4). The sediment $\delta^{15}N$ profile for Dart's Lake showed a general increase downcore from the sediment–water interface, especially in the top 10 cm (Fig. 4). In Arbutus Lake, sediment $\delta^{15}N$ values were more uniform with depth, with a small increase below 2 cm.

Sediment profiles of total N concentration were similar in both cores and showed a general decrease with depth, especially in Arbutus Lake (Fig. 4). Surface sediment C:N ratios also varied between the lakes (14.0 and 17.1 for Arbutus Lake and Dart's Lake, respectively) (Table 4).

Discussion

For marine systems, a negative relationship between ambient NO_3^- concentration and $\delta^{15}N$ values of plankton has been documented (Wada and Hattori 1976; Altabet 1989). Phytoplanktonic uptake of NO_3^- and the resulting isotopic fractionation explain these observations. Few previous studies in freshwater ecosystems have investigated the relationship between water column NO_3^- concentration and phytoplanktonic or seston $\delta^{15}N$.

Autochthonous production was likely a major source of material for our sediment traps based on the observed seston C:N ratios (Table 3). C:N ratios of autochthonous sources are generally much lower than those of allochthonous materials such as soils (LaZerte 1983; Hecky et al. 1993). Additional evidence is from potential terrestrial sources of organic materials in our study watersheds that differ in elemental and isotopic compositions of C and N from the seston in these lakes (Table 4). For example, although mean $\delta^{15}N$ values of overstory foliage were higher within the Dart's Lake watershed, the opposite pattern was shown for seston $\delta^{15}N$ values. In contrast to the trend shown by LaZerte (1983), the $\delta^{13}C$ values of foliage from both of these watersheds were lower than seston $\delta^{13}C$ values. Sediment

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Fig. 4. Sediment profiles of (A) $\delta^{15}N$ and (B) total N concentration in sediment cores from Arbutus Lake (●) and Dart's Lake (○).

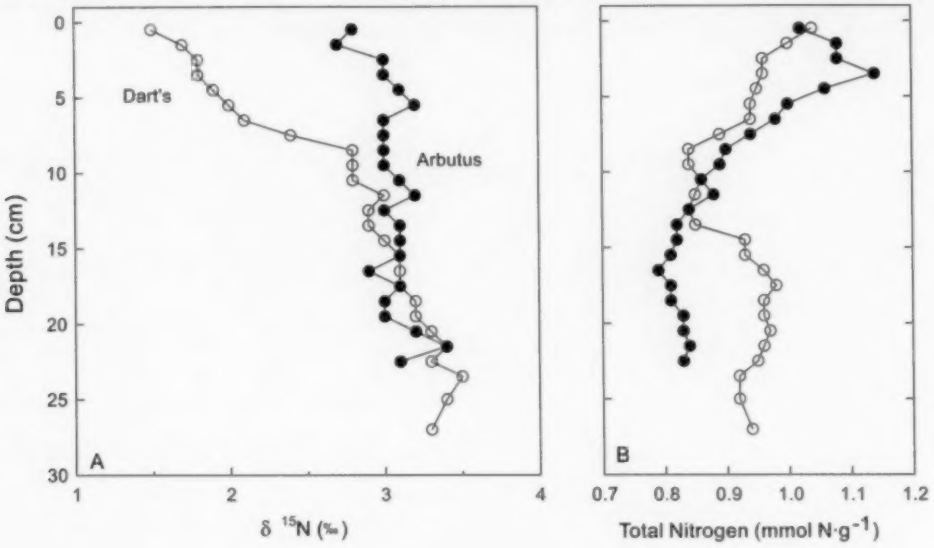
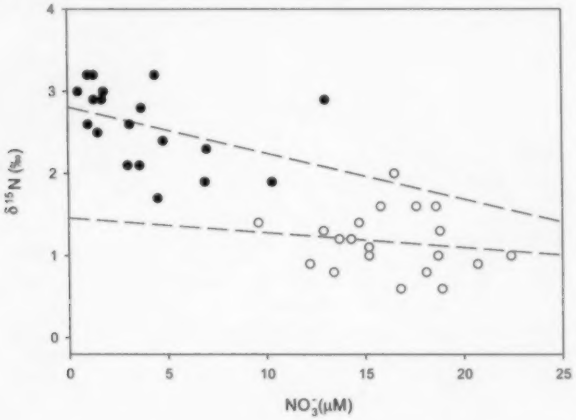


Fig. 5. Relationship between seston $\delta^{15}N$ values and water column NO_3^- concentration. The solid line is the regression line (p = 0.022) for Arbutus Lake (●); the broken line is the regression line (p = 0.492) for Dart's Lake (○).



resuspension is important in redistributing sediments in some lakes (Pennington 1974; Lastein 1976). The similarity between surface sediment $\delta^{15}N$ values (2.8‰) and seston $\delta^{15}N$ value maxima at the lower depth in Arbutus Lake during late September may indicate that algal production during the late summer period is a major contributor to sediment accumulation. The possibility that sediment may have been mixed into the water column during autumnal overturn cannot be discounted, however. Sediment in Dart's Lake (1.6‰), which is about twice as deep, was slightly enriched in ^{15}N relative to seston, indicating the possibility of post-depositional changes in isotopic composition occurring and (or) that sediment resuspension and accumulation in the sediment traps during overturn is not important in this lake.

The inverse relationship between oceanic NO_3^- concentration and particulate matter $\delta^{15}N$ has been described by a Raleigh relationship (Altabet and Francois 1994). Using data from both our study lakes, a linear relationship between NO_3^- concentration and seston $\delta^{15}N$ was clearly indicated (Fig. 5). Overall, for both lakes, the relationship was significant ($\delta^{15}N = -0.0997[NO_3^-] + 2.8780$, $r = -0.836$, SKT p = 0.0001, n = 39). For Arbutus Lake, a linear relationship between NO_3^- concentration and seston $\delta^{15}N$ was suggested ($\delta^{15}N = -0.0560[NO_3^-] + 2.8089$, $r = -0.392$, SKT p = 0.022, n = 19). For Dart's Lake, there was no relationship between NO_3^- concentration and seston $\delta^{15}N$ ($\delta^{15}N = -0.0180[NO_3^-] + 1.4564$, $r = -0.153$, SKT p = 0.492, n = 20).

To help interpret these patterns, we calculated a simple measure of the relative depletion of the spring NO_3^- pool within each depth by subtracting the midsummer NO_3^- concentration from the NO_3^- concentration just after ice-out (isothermal condition) as a fraction of the spring NO_3^- concentration (Table 5). The spring NO_3^- pool in Arbutus Lake was nearly totally consumed (78–94% was consumed), while in Dart's Lake, a much smaller proportion of NO_3^- was consumed (24–34%). During NO_3^- uptake, if the substrate (i.e., NO_3^-) pool is completely consumed, there is no net isotopic fractionation (Peterson and Fry 1987; Montoya 1994). The critical aspect of observations from marine systems is the importance of NO_3^- consumption as opposed to absolute NO_3^- concentration and the expected change in the isotopic composition of substrate and product during NO_3^- assimilation (Montoya 1994). Unfortunately, isotopic data for dissolved N species in the water column are not available to examine isotopic changes in substrate pools in these lakes.

Differences in hydrology might affect the temporal pattern of NO_3^- concentrations and therefore seston $\delta^{15}N$ values. For example, lake retention time should influence the amount of planktonic NO_3^- depletion. Retention time is an important factor in determining the production of in-lake alkalinity (Kelly et al. 1987). Relative to Arbutus Lake, Dart's Lake has a short retention time (0.05 years), typical of many Adi-

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Table 5. Summary of water column NO_3^- concentration and seston $\delta^{15}N$ for Arbutus and Dart's lakes during 1993.

Site	Depth (m)	Spring NO_3^- (μequiv.·L^{-1})	Midsummer NO_3^- consumption (%)	$\delta^{15}N$ (minimum to maximum) (‰)	NO_3^- (minimum to maximum) (μequiv.·L^{-1})
Arbutus Lake	2	12.6	94	1.7 to 3.2	0.0 to 10.3
	6	14.2	78	1.9 to 3.2	1.0 to 13.0
Dart's Lake	6	23.4	24	0.6 to 1.6	14.7 to 22.4
	14	23.6	34	0.6 to 1.3	12.9 to 18.9

rondack drainage lakes (Driscoll et al. 1991). The smaller consumption of epilimnetic NO_3^- in Dart's Lake (Table 5) may be partially related to its rapid flushing rate.

The higher C concentration of seston in Dart's Lake might reflect differences in allochthonous C sources. Hecky et al. (1993) found that the elemental composition of particulate matter in lakes with short retention times more closely resembles that in inflowing streams than that in lakes with longer retention times. Driscoll et al. (1989) indicated that soils in the southwestern Adirondacks (including the area near Dart's Lake) have higher C:N ratios than soils in the central Adirondacks. The widely different watershed to lake ratios (6 for Arbutus versus 207 for Dart's) could also relate to the difference in C concentration.

In some lakes, seston C:N ratio increases with lake depth, presumably due to organic matter mineralization and preferential loss of N (Pennington 1974). In our study, there were no significant differences ($p > 0.05$) in seston C or N concentration between depths. The lack of change in elemental composition of seston with depth could be interpreted as evidence for lack of mineralization during sinking (David and Mitchell 1985). However, an additional hypothesis may also explain this observation. In many Adirondack lakes, the euphotic zone (estimated as 2.4 times the Secchi disk reading, Baines and Pace 1994) may extend into the hypolimnion (Bukaveckas 1988), suggesting that processes such as N uptake, primary production, and mineralization occur throughout nearly the entire water column, therefore complicating interpretation.

An interesting question is whether sediment $\delta^{15}N$ profiles can provide information about past conditions in lakes. The seston elemental composition and isotopic composition in these two lakes were similar to those of surface sediments within each lake (Table 4). Surface sediment from both Dart's and Arbutus lakes was slightly enriched relative to average seston $\delta^{15}N$ values. The difference in isotopic composition ($\Delta\delta^{15}N$) between sediment and seston was greater in Dart's Lake (0.5‰) than in Arbutus Lake (0.2‰). Different rates of sediment microbial processes, especially denitrification, could possibly contribute to this pattern (Driscoll and Schafran 1984).

Changes in isotopic composition occurring during decompositional processes could obscure historical reconstructions, but some evidence is available to suggest that changes in organic matter $\delta^{15}N$ values during decomposition are relatively small (Nadelhoffer and Fry 1994).

If $\delta^{15}N$ values of phytoplankton and hence seston are influenced by NO_3^- utilization, then the decreases in sediment $\delta^{15}N$ toward the top of our sediment cores (most pronounced in Dart's Lake, Fig. 5) are consistent with the predicted pattern for these lakes, which have experienced a general increase in NO_3^- concentration. Long-term lake outlet monitoring studies indicated that in our study lakes, concentration increased between 1982 and 1991 (Driscoll and Van Dreason 1993). However, after this period, the temporal pattern varied, but NO_3^- concentrations in Dart's Lake remained higher than those in Arbutus Lake.

Intensive paleolimnological studies on Big Moose Lake, immediately upstream from Dart's Lake, indicated that a rapid decrease in surface water pH and alkalinity and an increase in Al concentration occurred post-1950, corresponding to a sediment depth of less than 10 cm (Charles 1984). Although no chronologies or paleolimnological reconstructions are available for our study lakes, the upper 10 cm of sediment should represent post-1950 sediment accumulation. The present-day alkalinity (about 40–100 μequiv.·L^{-1}) of Arbutus Lake suggests that it is unlikely to have been acidic. Historical water chemistry records can be of uncertain quality, and paleolimnological methods offer a valuable long-term record of environmental change (e.g., Dixit et al. 1992). We recognize that additional research is needed regarding questions related to postdepositional changes in isotopic composition. We would not expect different profiles with sediment depth if isotopic fractionation during decomposition were operating similarly in these two lakes.

The inverse relationship between $\delta^{15}N$ values of particulate matter and NO_3^- utilization in marine systems has been well documented but has not been thoroughly investigated for freshwater systems. The most likely cause of the different relationship between NO_3^- concentration and isotopic composition of seston at these study sites is greater relative utilization of NO_3^- in Arbutus Lake compared with Dart's Lake (Fig. 4; Table 5). Interestingly, a greater range of seston $\delta^{15}N$ values was found for Arbutus Lake, the lake with the greater range of NO_3^- concentrations (Table 5).

We suggest that additional investigation of the patterns of stable N isotope distribution in freshwater systems is needed. If seston $\delta^{15}N$ values are recorded in sediment $\delta^{15}N$ values and the apparent inverse relationship between seston $\delta^{15}N$ values and lake water NO_3^- utilization can be further documented in freshwater systems, then a unique paleolimnological tool for investigating the historical N status of lakes may exist. Data on the stable isotopic composition of sediment, seston, and dissolved N species from a variety of lakes are needed to better understand the biogeochemical linkages between lakes and their watersheds.

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Effects of northern pike (*Esox lucius*) additions on pollutant accumulation and food web structure, as determined by $\delta^{13}C$ and $\delta^{15}N$, in a eutrophic and an oligotrophic lake

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Abstract: In May 1993 and 1994, northern pike (*Esox lucius*) were added to eutrophic Lake 227 and oligotrophic Lake 110 at the Experimental Lakes Area in northwestern Ontario. Both lakes were previously dominated by cyprinids, and northern pike additions significantly decreased cyprinid densities in both lakes. Food web relationships were determined pre- and post-manipulation using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses of zooplankton, benthic invertebrates, and fishes. In Lake 110, fathead minnow (*Pimephales promelas*) shifted from a zooplanktivorous to a zoobenthivorous diet as indicated by a shift in isotopic composition to more depleted $\delta^{15}N$ and enriched $\delta^{13}C$ values after northern pike additions. It was not possible to direct predator-induced shifts in cyprinid diets in Lake 227. Concentrations of mercury and organochlorines (sum of polychlorinated biphenyls (ΣPCB), dichlorodiphenyltrichloroethane (ΣDDT), and hexachlorocyclohexane (ΣHCH)) in these fish did not change markedly after northern pike introductions despite the shifts in diet for fathead minnow from Lake 110. However, concentrations of all contaminants were lower in biota from eutrophic Lake 227 when compared with Lake 110, which is consistent with previous studies. Our results indicate that nutrient availability, rather than short-term changes in food web structure, determined contaminant concentrations in fish and other biota from these lakes.

Résumé : Aux mois de mai 1993 et 1994, on a introduit des grands brochets (*Esox lucius*) dans le lac eutrophe 227 et le lac oligotrophe 110 de la Région des lacs expérimentaux du nord-ouest de l'Ontario. Dans les deux lacs, les cyprinidés dominaient avant les introductions des grands brochets; après celles-ci, les densités de cyprinidés ont diminué significativement dans les deux lacs. On a déterminé les relations trophiques avant et après introduction des grands brochets par l'analyse des isotopes stables de carbone ($\delta^{13}C$) et d'azote ($\delta^{15}N$) dans le zooplancton, les invertébrés benthiques et les poissons. Dans le lac 110, les têtes-de-boules (*Pimephales promelas*) sont passées d'un régime zooplanctivore à un régime zoobenthivore, comme l'a indiqué le changement dans la composition isotopique, soit une baisse de $\delta^{15}N$ et une hausse de $\delta^{13}C$, après l'introduction des grands brochets. On n'a pas pu détecter de changements induits par les prédateurs dans le régime des cyprinidés dans le lac 227. Les concentrations de mercure et d'organochlorés (la somme de polychlorobiphényles (ΣPCB), de dichlorodiphényltrichloroéthane (ΣDDT) et de hexachlorocyclohexane (ΣHCH)) dans ces poissons n'ont pas changé de façon marquée après les introductions de grands brochets, malgré la modification du régime alimentaire des têtes-de-boules dans le lac 110. Cependant, les concentrations de tous les contaminants étaient plus faibles dans le biote du lac eutrophe 227 que dans celui du lac 110, ce qui va dans le sens des études antérieures. Nos résultats indiquent que c'est la disponibilité des nutriants, plutôt que les changements à court terme dans la structure du réseau alimentaire, qui a déterminé les concentrations de contaminants dans les poissons et les autres organismes de ces lacs.

[Traduit par la Rédaction]

Introduction

Several studies have found large variations in organochlorine (e.g., polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT)) and mercury (Hg) concentrations in fish from lakes receiving similar contaminant loadings (Larsson et al. 1992; Bentzen et al. 1996). Evidence is accumulating that much of this variation might be explained by differences in food web structure and trophic conditions. Top predators from lakes with longer un-

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Table 1. Physical and chemical characteristics of lakes 110 and 227, ELA, northwestern Ontario.

Characteristics	Lake 110	Lake 227
Watershed area (ha)	34.0	34.4
Lake area (ha)	5.3	5.0
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	3.2	25.7
Suspended carbon ($\mu g \cdot L^{-1}$)	921.7	2774.0
Dissolved organic carbon ($\mu mol \cdot L^{-1}$)	576.7	868.6
pH	6.6	7.5
^{14}C primary production (mg $C \cdot m^{-2} \cdot day^{-1}$)	201.8	742.7

Note: Water chemistry values are means for biweekly samples collected from the epilimnion between May and October 1993.

derlying food webs (Kidd et al. 1995; Bentzen et al. 1996) and biota from less productive lakes (Taylor et al. 1991; Larsson et al. 1992) have higher pollutant concentrations than the same organisms from contrasting systems. Species introductions have also affected contaminant concentrations in top predators by lengthening the food web; for example, higher concentrations of organochlorines and Hg have been found in lake trout (*Salvelinus namaycush*) from lakes containing the exotic species smelt because this forage fish feeds at a higher trophic level than other pelagic fishes (Vander Zanden and Rasmussen 1996).

Many lakes (e.g., the Laurentian Great Lakes) have been subjected to perturbations such as nutrient additions and reductions, fish stocking, and introductions of exotic species. This temporal variability in production and community composition will affect not only pathways of energy and nutrient transfer from primary consumers to top predators, but also, potentially, the food web transfer of contaminants. Hence, an understanding of interactions among food web structure, trophic status, and contaminant cycling is essential to guide efforts to reduce contaminant concentrations in aquatic biota.

Manipulations of freshwater food webs, through either species introductions or nutrient additions, have frequently been used to elucidate factors controlling productivity, population biomass, and community structure in lakes. Cascading trophic interactions (e.g., decreases in planktivorous fishes, increases in zooplankton, and decreases in phytoplankton) have been observed in many systems that have been perturbed through the addition of piscivorous fishes (e.g., Carpenter and Kitchell 1993). Piscivore introductions also often affect interspecific competition within trophic levels, the quantity of resources available to consumers, and the behaviour and diet of prey populations (e.g., Carpenter and Kitchell 1993).

Recent advances in understanding trophic interrelationships of aquatic biota and contaminant accumulation through food webs have been made using measurements of stable carbon and nitrogen isotopes. Standardized ratios of heavier to lighter carbon ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) isotopes characterize the relative trophic positioning of biota within a food web by identifying both the source of carbon in an organism's diet (Fry and Sherr 1984) and its trophic level (Cabana and Rasmussen 1994), respectively. The heavier isotope of nitrogen is progressively enriched about 3‰ with each trophic level from primary producers up to top predators (Peterson and Fry 1987) and provides a nondiscrete measure of an individual's relative trophic position within a food web. In contrast with nitrogen, little or no fractionation of carbon isotopes occurs as energy is transferred from prey to predator (0 to 1‰; Peterson and Fry 1987), and these ratios are used to identify sources of carbon that are important to an organism's growth (Hecky and Hesslein 1995). In most temperate lakes, benthic and pelagic primary producers and the primary consumers relying upon these carbon sources can be distinguished due to their heavier and lighter $\delta^{13}C$ signals, respectively (Hecky and Hesslein 1995). For these reasons, biotic stable isotope analyses may prove to be an important tool in understanding the impacts of whole-lake or mesocosm manipulations on the dietary habits of organisms at all levels of the food web.

The objectives of this study were to examine how alterations in food web structure, through piscivorous fish additions, and differences in nutrient loadings affected food web relationships (as indicated by $\delta^{15}N$ and $\delta^{13}C$) and concentrations of PCBs, hexachlorocyclohexane (HCH), DDT, and Hg in biota from two lakes (lakes 110 and 227) at the Experimental Lakes Area (ELA) in northwestern Ontario. It was expected that the introduction of a piscivore would initiate cascading trophic interactions and that this in turn might affect contaminant concentrations in biota through changes in dietary habits or growth rates of prey species.

Materials and methods

Study design and sample collection

Lake 110 is naturally oligotrophic whereas Lake 227 is an artificially eutrophic system that has received nutrient additions since 1968 (Table 1). Between 1990 and the present, the lake has been treated with phosphorus at rates of 0.56 g $P \cdot m^{-2} \cdot year^{-1}$ (Findlay et al. 1994). Both lakes are remote from point source inputs of contaminants and were chosen for a comparative study because of their similar physical characteristics (Table 1). Prior to food web manipulations, lakes 227 and 110 lacked piscivorous fish and were dominated by populations of cyprinids. Lake 227 contained fathead minnow (*Pimephales promelas*) and northern redbelly dace (*Phoxinus eos*) whereas the fish community of Lake 110 was more diverse and included fathead minnow, northern redbelly dace, finescale dace (*Phoxinus neogaeus*), pearl dace (*Margariscus margarita*) (rare), and white sucker (*Catostomus commersoni*) (rare). Both fathead minnow and pearl dace are known to feed upon algae or detritus, zooplankton, and aquatic insects (Scott and Crossman 1973). At ELA, fathead minnow in one lake fed mainly upon cladocerans (33–53% of stomach contents) and detritus (30–36% of stomach contents) (Tallman et al. 1984).

Northern pike (*Esox lucius*) from three nearby lakes (lakes 222, 663, and 240) were added to lakes 227 and 110 in May 1993 (60 and 56 fish, respectively) and May 1994 (131 and 124 fish, respectively), resulting in corresponding predator densities of 9.2 and 8.1 $kg \cdot ha^{-1}$ (George 1994; Elser et al. 1998).

Fathead minnow, northern redbelly dace, and several invertebrate taxa were collected from each lake pre- and post-manipulation and analysed for $\delta^{13}C$ and $\delta^{15}N$, organochlorines, and Hg. Fathead minnow and northern redbelly dace were collected in May 1992 (fathead minnow only), May and September 1993, and May and (or) June 1994 using Fyke nets located at a water depth of 1–3 m. Some individuals of northern redbelly dace were difficult to separate from finescale dace in Lake 110 and may have been mistakenly identified. Northern pike were collected using gill nets (Elser et al. 1998). All fish were measured and weighed after capture. For each cyprinid species, a minimum of 10 fish, representing a range of sizes, was selected for Hg analyses from each lake on each sam-

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Table 2. $\delta^{13}C$ and $\delta^{15}N$ of lower trophic level biota from Lake 110, ELA, northwestern Ontario.

Order	Family/species/other	Date collected	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
	Epiphyton	August 1993	–24.69	0.98
	Epilithiphyton	August 1993	–22.50	2.27
Amphipoda	*Hyallela azteca*	May 1993	–28.57	—
Annelida	Leech	May 1993	–28.54	10.31
Coleoptera	*Dineutus* sp.	June 1993	–27.49	3.84
	Gyrinus sp.	June 1993	–31.08	3.45
Diptera	*Chaoborus* spp.	Summer 1993	–33.76±1.44	9.46±1.01
	Chironomidae	May 1993	–29.63	4.25
	Tanypodinae	May 1993	–27.91	3.68
Ephemeroptera	*Hexagenia* sp.	May 1993	–30.07	1.87
	Caenis sp.	June 1993	–29.35	—
Hemiptera	*Gerris* sp.	June 1993	–28.23	3.52
	Hesperocorixa sp.	June 1993	–26.37	4.53
Lepidoptera	*Anabolia bimaculata*	May 1993	–28.04	3.29
Odonata	*Aeshna eremita*	June 1993	–29.34	5.37
	Aeshna eremita	June 1993	–28.88	4.50
	Chromagrion conditum	June 1993	–28.67	4.87
	Cordulia shurtleffi	June 1993	–28.55	3.75
	Dromogomphus spinosus	June 1993	–27.08	4.18
	Enallagama nayanilebrirum	June 1993	–29.59	5.40
	Libellulidae	June 1993	–28.24	2.65
	Macromia illinoiensis	May 1993	–27.85	3.77
Spongillidae	*Spongilla lacustris*	August 1993	–30.62	4.86
Trichoptera		June 1993	–30.82	4.60
	Zooplankton	Summer 1993	–33.94±2.92	8.36±1.81
	POM	Summer 1993	–33.81±4.57	2.94±1.84

Note: Values are means ± SD for *Chaoborus* spp., zooplankton, and POM from sediment traps.

pling date. Cyprinids were also combined into species-specific samples of 8–143 fish to obtain sufficient biomass for organochlorine analyses. Samples of fathead minnow collected in 1993 and 1994 were selected from two dominant size-classes and grouped by size on each sampling date. Zooplankton were collected monthly from May to October 1993 by towing a 50-cm-diameter net with a 150-μm mesh behind a boat. *Bosmina longirostris*, *Diaphanosoma birgei*, *Epischura lacustris*, and *Mesocyclops edax* dominated the crustacean zooplankton community of Lake 227. Common zooplankton taxa in Lake 110 included *Leptodiaptomus minutus*, *E. lacustris*, and *Daphnia retrocurva* (Elser et al. 1998; Sterner 1998). Samples composed primarily of *Chaoborus* spp. were collected by vertical net tows made at least 1 h after sunset and then passed through a 400-μm sieve. Littoral invertebrates were collected using dip nets and sorted and pooled by species whenever possible. All invertebrate samples were frozen in glass jars precleaned with acetone. Sediment traps were deployed in each lake below the epilimnion and sedimented particulate organic matter (POM) was collected biweekly from mid-June to mid-October 1993 (Jeremiason et al. 1999). Microscopic inspection of sediment trap samples revealed that they were composed almost exclusively of phytoplankton remains (H. Kling, Freshwater Institute, Winnipeg, Man., personal communication).

Stable isotope analysis

Invertebrates were removed from shells or cases if present and pooled to obtain adequate masses for analyses (one to five individuals for macroinvertebrates). Skinless dorsal muscle tissue from individual northern pike, fathead minnow (except a June 1992 composite sample of whole fish from both lakes), and northern redbelly dace and whole invertebrate and sediment trap samples were dried in an oven at 60°C and ground to a fine powder using a mortar and pestle. After subsampling for nitrogen isotope analyses, invertebrate and sediment trap samples were treated with 1 N HCl to remove carbonates. Tissue samples (1–4 mg) were combusted and analysed on a VG Micromass 602E using the methods detailed in Kidd et al. (1998). All samples were standardized against N_2 in air or CO_2 in PeeDee limestone as follows:

$$\delta^{15}N \text{ or } \delta^{13}C \text{ (‰)} = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$. A laboratory working standard of Pharmamedium was run with every 10 samples. Precision of these instruments for nitrogen and carbon isotope analysis over several years of use has been 0.4 and 0.3‰ (2 SD), respectively.

Organochlorine analyses

Composite samples of fathead minnow and northern redbelly dace were homogenized with dry ice. Zooplankton and other invertebrate samples were blotted to remove surficial water prior to extraction. Tissues were combined with anhydrous Na_2SO_4 (heated at 600°C for 16 h prior to use) and internal standards of aldrin (or PCB30) and octachloronaphthalene were added prior to extraction. Fish and invertebrates were Soxhlet extracted, lipids were removed and quantified, and extracts were fractionated and analysed using the methods and quality assurance procedures detailed in Kidd et al. (1998). Average recoveries of internal standards for all samples were between 80 and 87% for PCB30 or aldrin and octachloronaphthalene, respectively. No correction was made for recovery efficiencies. Three main organochlorine groups were quantified in these samples: ΣPCB was the sum of all congeners present in the biota (see Kidd et al. (1998) for list of congeners), ΣDDT was the sum of *p,p′*- and *o,p′*-DDT, *p,p′*- and *o,p′*-DDE, and *p,p′*- and *o,p′*-DDD, and ΣHCH was the sum of α-HCH, β-HCH, δ-HCH, and γ-HCH. Minimum detectable levels were calculated as described in

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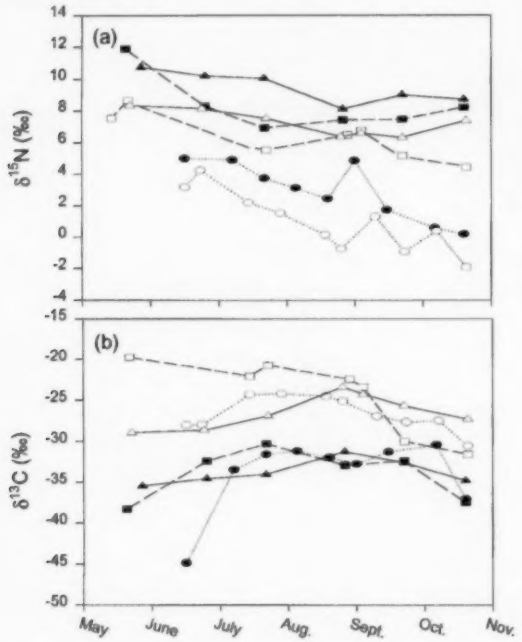
Fig. 1. (*a*) $\delta^{15}N$ and (*b*) $\delta^{13}C$ of sediment trap material (circles), bulk zooplankton (squares), and *Chaoborus* spp. (triangles) collected from May to October 1993 from oligotrophic Lake 110 (solid symbols) and eutrophic Lake 227 (open symbols), ELA, northwestern Ontario.



Long and Winefordner (1983) and averaged 0.02 $ng \cdot g^{-1}$ for individual tri- to octa-chlorobiphenyls and most organochlorine pesticides.

Mercury analyses

Total Hg concentrations were determined in individual whole cyprinids (after removal of stomachs) using cold-vapour atomic absorption spectrophotometry and the method of Armstrong and Uthe (1971). For zooplankton, methyl mercury (MeHg) was measured in freeze-dried samples using the methods described in Paterson et al. (1998). We measured MeHg in zooplankton because this form of Hg predominates in fish (Bloom 1992). For all statistical analyses, we have used a significance level of 0.05.

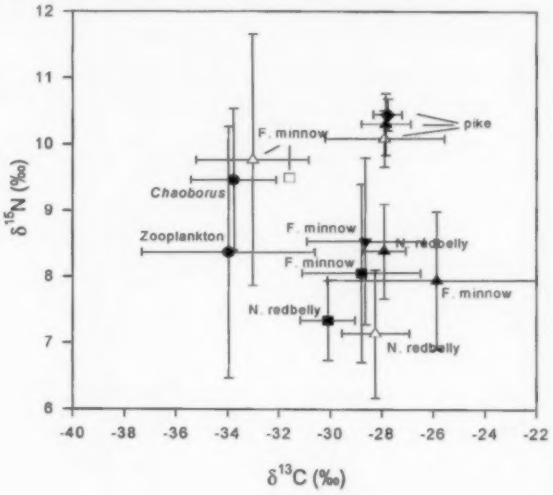
Results

Food web relationships before and after northern pike additions

Lake 110

The stable isotopic composition of pelagic invertebrates and POM from Lake 110 varied over the summer season and was distinct from that of benthic invertebrates. Planktonic invertebrates were more depleted in ^{13}C ($\delta^{13}C$ = –38.3 to –30.3‰) and more enriched in ^{15}N ($\delta^{15}N$ = 6.9 to 11.9‰) when compared with most of the benthic invertebrates collected over the same period ($\delta^{13}C$ = –31.08 to –26.37‰ and $\delta^{13}N$ = 1.87 to 5.40‰) (Table 2). The $\delta^{13}C$ values of the pelagic biota ranged by up to 15‰ over the sampling period and were generally depleted in May and in the fall and were enriched in midsummer (Fig. 1). In May and June 1993, the

Fig. 2. Mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ of fathead minnow (n = 6–12), northern redbelly dace (n = 3–12), zooplankton (n = 6), and *Chaoborus* (n = 6) before (□, May 1992; △, May 1993) and after (●, summer 1993; ▲, September 1993; ■, May 1994; ▼, June 1994; ◆, September 1994) the addition of northern pike to oligotrophic Lake 110, ELA, northwestern Ontario.



$\delta^{13}C$ values of zooplankton, *Chaoborus*, and POM (representing the previous 2 weeks of deposition) were distinct from each other; however, samples collected later in the summer (August–October) had comparable $\delta^{13}C$ values. The $\delta^{15}N$ values of POM, *Chaoborus*, and zooplankton were highest in May and became progressively lighter through the summer (Fig. 1). *Chaoborus* were occasionally at a higher trophic level than zooplankton, as indicated by the magnitude of difference in their $\delta^{15}N$ values at each sampling date ($\delta^{15}N_{Chaoborus} - \delta^{15}N_{zooplankton}$ = 0.7 to 3.12‰).

Before northern pike were added to Lake 110, mean $\delta^{13}C$ (–33.02‰) and $\delta^{15}N$ (9.76‰) of fathead minnow were consistent with a diet of zooplankton (mean $\delta^{13}C$ = –33.95‰ and mean $\delta^{15}N$ = 8.39‰) and possibly some POM (mean $\delta^{13}C$ = –33.81‰ and mean $\delta^{15}N$ = 2.94‰), given that fathead minnow $\delta^{15}N$ was lower than would be predicted from a diet of zooplankton alone (Fig. 2; Table 1). In contrast, the isotopic composition of northern redbelly dace (mean $\delta^{13}C$ = –28.25 and mean $\delta^{15}N$ = 7.14‰) indicated that this species fed primarily upon littoral benthic invertebrates such as the dipterans (Table 2; Fig. 2).

After northern pike additions, fathead minnow were enriched in $\delta^{13}C$ (mean = –25.87‰) and depleted in $\delta^{15}N$ (mean = 7.94‰) when compared with individuals of the same species collected in the spring of 1993 (Fig. 2). This change in isotopic composition indicated that fathead minnow shifted from a planktivorous to a zoobenthivorous diet after food web manipulations. Mean $\delta^{15}N$ values of northern redbelly dace increased from 7.14 to 8.38‰, but no change was observed in their $\delta^{13}C$ values in the fall after northern pike additions; in addition, the isotopic composition of this species in the fall of 1993 and the spring of 1994 continued to be consistent with a diet of zoobenthos (Fig. 2; Table 2).

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Table 3. $\delta^{13}C$ and $\delta^{15}N$ of lower trophic level biota from Lake 227, ELA, northwestern Ontario.

Order	Family/species/other	Date collected	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
	Epiphyton	August 1993	–27.23	0.73
	Epilithiphyton	August 1993	–24.89	3.44
Amphipoda	*Hyallela azeteca*	September 1992	–23.57	3.85
	Hyallela azeteca	May 1993	–26.29	2.88
	Cragonyx sp.	May 1993	–25.02	7.00
Annelida	Leech sp. 1	May 1993	–26.23	9.47
	Leech sp. 2	May 1993	–27.08	5.61
	Leech sp. 3	May 1993	–26.10	6.19
Coleoptera	*Dineutus* sp.	September 1992	–25.79	2.80
	Dineutus sp.	May 1993	–26.26	2.93
	Haliplus sp.	September 1992	–28.52	2.17
	Haliplus sp.	May 1993	–31.26	0.63
	Gyrinus sp.	September 1992	–24.41	3.71
	Gyrinus sp.	May 1993	–24.92	4.44
Diptera	Ceratopogonidae	May 1993	–26.49	3.57
	Chaoborus spp.	Summer 1993	–26.40±2.11	7.23±0.84
	Chironomidae	September 1992	–27.27	2.24
	Chironomidae	May 1993	–29.39	2.73
	Tabanidae	September 1992	–24.44	3.75
	Tabanidae	May 1993	–28.27	3.31
	Tanypodinae	May 1993	–27.25	4.19
Ephemeroptera	*Leptophlebia* sp.	September 1992	–25.43	—
	Unknown sp.	May 1993	–22.65	—
Gastropoda	Planorbidae	September 1992	–27.42	3.26
	Planorbidae	May 1993	–23.07	3.93
Hemiptera	*Belostoma* sp.	September 1992	–24.83	3.40
	Notonecta sp.	September 1992	–36.08	4.91
	Notonecta sp.	May 1993	–29.56	5.79
	Hesperocorixa sp.	September 1992	–25.83	4.39
	Hesperocorixa sp.	May 1993	–24.82	4.41
	Ranatra sp.	September 1992	–25.40	3.71
Odonata	*Aeshna interrupta*	May 1993	–24.74	4.60
	Aeshna interrupta	May 1993	–26.20	4.99
	Aeshna sp.	September 1992	–28.12	4.04
	Cordullia shurtleffi	May 1993	–25.41	2.48
	Enallagma sp.	September 1992	–25.94	3.05
	Enallagma sp.	May 1993	–26.48	4.99
	Epitheca cynogera	May 1993	–26.57	3.13
	Gomphus spicatus	May 1993	–25.73	3.69
	Libellulidae	September 1992	–25.78	4.19
	Libellula julia	May 1993	–25.29	3.51
Spongillidae		August 1993	–27.21	2.08
Trichoptera	*Phryganea* sp.	September 1992	–25.22	2.81
	Limnephilus sp.	May 1993	–23.15	4.86
	Unknown sp.	May 1993	–26.02	4.73
	Zooplankton >20 µm	September 1992	–27.00	4.25
	Zooplankton 80–200 µm[a]	September 1992	–26.95	3.31
	Zooplankton 150 µm	Summer 1993	–24.25±4.61	6.36±1.46
	POM	Summer 1993	–26.63±2.08	0.94±1.92

Note: Values are means ± SD for *Chaoborus* spp., zooplankton, and POM from sediment traps.
[a]Contains blue-green algae.

Lake 227

As was observed in Lake 110, the $\delta^{13}C$ and $\delta^{15}N$ of the pelagic invertebrates and POM varied over the summer; $\delta^{13}C$ values of zooplankton, *Chaoborus*, and POM from Lake 227 were lightest in early summer and late fall and heaviest in August and September (Fig. 1). Temporal changes in the $\delta^{13}C$ composition of *Chaoborus* lagged behind those of POM but were similar in direction and magnitude. On half of the sampling dates, *Chaoborus* appeared to be feeding at the same trophic level ($\delta^{15}N$) as zooplankton (Fig. 1). This

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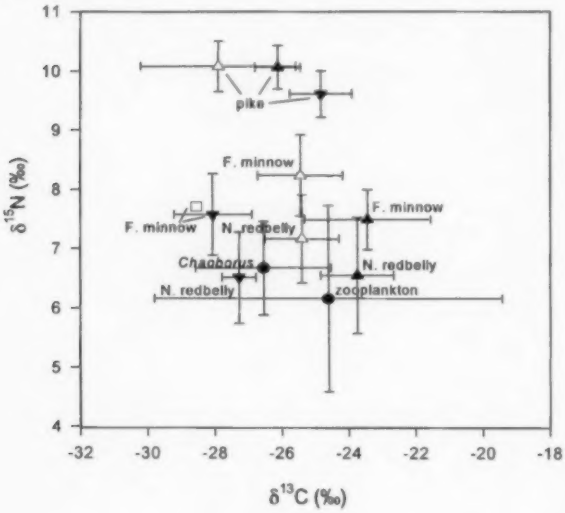
Fig. 3. Mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ of fathead minnow (n = 6–12), northern redbelly dace (n = 3–12), zooplankton (n = 6), and *Chaoborus* (n = 6) before (□, June 1992; △, May 1993) and after (●, summer 1993; ▲, September 1993; ▼, June 1994) the addition of northern pike to eutrophic Lake 227, ELA, northwestern Ontario.



may be because our zooplankton samples were dominated by *E. lacustris*, a predatory copepod (M.J. Paterson, unpublished data). Both *E. lacustris* and *Chaoborus* may have fed mainly upon rotifers that were too small to be collected in our nets. Unlike Lake 110, benthic bottom-dwelling invertebrates collected from Lake 227 had $\delta^{13}C$ values that overlapped (–23.1 to –31.3‰) with those of zooplankton (–19.7 to –31.5‰) (Table 3). For this reason, it was not possible to use $\delta^{13}C$ data to delineate the feeding habits of cyprinids in this lake. With the exceptions of leeches and one species of amphipod, the $\delta^{15}N$ values of bottom-dwelling invertebrates were generally depleted by 2 to 3‰ when compared with zooplankton collected during the same summer season (Table 3).

Absolute values of $\delta^{13}C$ for POM, zooplankton, and *Chaoborus* were more enriched in Lake 227 (–31.5 to –19.7‰) than in Lake 110 (–44.8 to –30.3‰) but exhibited the same temporal trends in both systems (Fig. 1). In addition, POM, zooplankton, and *Chaoborus* were more enriched in $\delta^{15}N$ in Lake 110 when compared with the same respective components of the Lake 227 food web, and the seasonal $\delta^{15}N$ changes in these fractions were also similar in magnitude and direction in both lakes (Fig. 1).

Prior to the addition of northern pike to Lake 227, fathead minnow (mean $\delta^{15}N$ = 8.24‰) fed mainly upon zooplankton (mean $\delta^{15}N$ = 6.36‰) and not upon POM (mean $\delta^{15}N$ = 0.94‰), *Chaoborus* (mean $\delta^{15}N$ = 7.23‰), or benthic invertebrates (mean $\delta^{15}N$ = 3.85‰, excluding leeches, n = 30) (Table 3). Northern redbelly dace had lighter $\delta^{15}N$ values (mean = 7.17‰) when compared with fathead minnow and suggests that northern redbelly dace relied mainly upon bottom-dwelling invertebrates (Table 3).

Given the similarity in $\delta^{13}C$ values of the benthic and pelagic invertebrates in Lake 227, it was not possible to determine whether northern pike additions affected the diets of cyprinids. Fathead minnow and northern redbelly dace were more enriched and depleted in $\delta^{13}C$ and $\delta^{15}N$, respectively, in the fall when compared with the same species collected the previous spring (Fig. 3). These changes for fathead minnow between May and September 1993 were similar in magnitude and direction to those observed for zooplankton over the same time (Figs. 1 and 3). This suggests that the fathead minnow continued to rely upon zooplankton after northern pike were added. However, seasonal differences in the isotopic composition of benthic invertebrates were also observed, although some of these shifts did not parallel those observed in zooplankton (Table 3).

We used the difference between mean $\delta^{15}N$ of fathead minnow and seasonal mean $\delta^{15}N$ of zooplankton to determine whether the relative trophic position of this species differed between the two lakes and varied after northern pike additions. To make this calculation, we assumed that mean $\delta^{15}N$ values of zooplankton samples collected between May and October were comparable with their annual mean $\delta^{15}N$ and isotopically comparable with the zooplankton consumed by fathead minnow before May 1993. Prior to northern pike additions, fathead minnow in Lake 110 were at a slightly lower trophic position ($\delta^{15}N_{minnows} - \delta^{15}N_{zooplankton}$ = 1.4‰) when compared with the same species from Lake 227 ($\delta^{15}N_{minnows} - \delta^{15}N_{zooplankton}$ = 1.9‰) and with northern redbelly dace ($\delta^{15}N_{dace} - \delta^{15}N_{invertebrates}$ = 3.0‰; benthic invertebrates with $\delta^{13}C$ from –30 to –22‰, excluding leeches, had a mean $\delta^{15}N$ of 4.1‰; Table 2) collected at the same time from Lake 110. In September 1993 after food web manipulations, the absolute difference between fathead minnow or northern redbelly dace from Lake 110 and their prey of benthic invertebrates was 3.8 and 4.3‰, respectively. These isotopic data suggest that both fish species occupied a higher relative trophic position after northern pike were added to Lake 110.

Organochlorines and Hg in biota

Cyprinids

There were no statistically significant differences in ΣPCB, ΣDDT, or ΣHCH concentrations in different size-classes of fathead minnow or between the two species of cyprinids in each lake (paired t tests, $p > 0.3$). Hence, we combined data for the different species and size-classes to facilitate comparisons between lakes and among dates. Average coefficients of variation for duplicate analyses of ΣHCH, ΣDDT, and ΣPCB in the cyprinid samples were less than 10%.

Concentrations of ΣPCB and ΣDDT in cyprinids were significantly higher in oligotrophic Lake 110 than in eutrophic Lake 227 (paired t tests, $p < 0.01$) (Fig. 4). On average, concentrations of ΣPCB and ΣDDT in cyprinids were twofold higher in Lake 110 than in Lake 227. No significant differences in the concentrations of ΣHCH in cyprinids were observed between the two lakes (paired t test, $p > 0.35$). There were no large temporal changes in concentrations of organochlorine contaminants (Fig. 4), despite changes in abundances (see Discussion) and feeding habits of fathead minnow (Lake 110 only) (Fig. 2).

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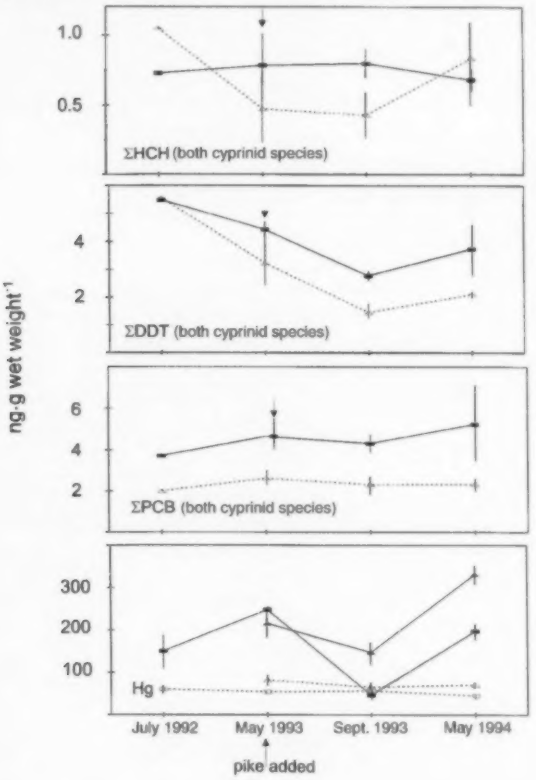
Fig. 4. Mean (± SE) concentrations of HCH, DDT, PCBs (■, Lake 110; △, Lake 227; n = 3 or 4 for 1993 and 1994; n = 1 for 1992 samples), and total Hg (squares, fathead minnow; triangles, northern redbelly dace; solid symbols, Lake 110; open symbols, Lake 227; n = 10) in two species of cyprinids (fathead minnow and northern redbelly dace) from oligotrophic Lake 110 and eutrophic Lake 227, ELA, northwestern Ontario (organochlorine data were combined for both cyprinid species).



With one exception (Lake 227 in September 1993), concentrations of Hg in northern redbelly dace and fathead minnow were not significantly correlated with either length or weight. On all but one date (May 1993), northern redbelly dace had significantly higher concentrations of Hg than fathead minnow within each lake. Both fathead minnow and northern redbelly dace were significantly higher in Hg in oligotrophic Lake 110 when compared with the same species from eutrophic Lake 227 for all but the fall 1993 samples (Fig. 4). Concentrations of Hg in fathead minnow from Lake 110 declined markedly in September 1993 and were lower than concentrations observed in the same species from Lake 227.

Invertebrates

Organochlorine concentrations were examined for limited numbers of zooplankton, *Chaoborus*, Odonata, and Hirudinea collected from lakes 110 and 227 (Table 4). Concentrations of ΣHCH, ΣDDT, and ΣPCB in zooplankton were not statistically different between lakes 110 and 227 (paired t tests, $p < 0.05$). On all dates, concentrations of MeHg were higher in zooplankton from oligotrophic Lake 110 (mean = 146 ± 6.4 ng·g dry weight^{-1}) than in zooplankton from eutrophic Lake 227 (mean = 35.3 ± 8.8 ng·g dry weight^{-1}). There were no systematic changes in zooplankton MeHg concentrations over time in either lake. Sample sizes were too small to permit statistical comparison of contaminant concentrations in macroinvertebrates; however, with one exception, levels of ΣDDT and ΣPCB were highest in *Chaoborus*, Odonata, and Hirudinea from oligotrophic Lake 110.

Food webs

On a wet weight basis, concentrations of organochlorines were significantly related to lipid content when zooplankton, *Chaoborus*, and fathead minnow were considered together (e.g., log ΣDDT versus log lipid: Lake 110, $p < 0.005$, n = 8, r^2 = 0.88; Lake 227, $p < 0.001$, n = 12, r^2 = 0.69). No relationships between organochlorine concentrations and lipid were found for individual species ($p < 0.05$).

Discussion

Food web relationships using $\delta^{13}C$ and $\delta^{15}N$

Unlike gut content analyses, stable carbon and nitrogen isotope ratios reflect the dietary history of an organism over an integrated period that is dependent on its growth rate and tissue turnover time (Hesslein et al. 1993). Lacustrine biota with shorter life spans exhibit more annual and seasonal variability in $\delta^{13}C$ and $\delta^{15}N$ composition than longer-lived organisms, and this temporal variability can be up to 10-fold higher for small pelagic biota than for the larger upper trophic level organisms (Zohary et al. 1994; Cabana and Rasmussen 1996). In this study, we observed temporal changes of up to 14‰ in the $\delta^{13}C$ of POM at the base of the pelagic food web; however, seasonal changes in the $\delta^{13}C$ and $\delta^{15}N$ of longer-lived organisms (e.g., *Chaoborus*) were not of the same magnitude and lagged behind changes occurring at lower trophic levels (Fig. 1). These factors need to be considered when interpreting the instantaneous $\delta^{13}C$ and $\delta^{15}N$ composition of prey and consumers. This variability in $\delta^{13}C$ and $\delta^{15}N$ in lower trophic level biota can confound interpretations of food web relationships if seasonal patterns are not considered and may result in an erroneous estimation of the relative importance of prey species in a predator's diet.

In lakes 227 and 110, POM, zooplankton, and *Chaoborus* were lightest in $\delta^{13}C$ in the spring and fall and heaviest in midsummer (Fig. 1), and this variability was similar to what has been observed in other studies (Yoshioka et al. 1994; Zohary et al. 1994). Zohary et al. (1994) found that $\delta^{13}C$ in pelagic biota of Lake Kinneret was most depleted at times of physical mixing and most enriched during periods of lake stratification. The depleted ^{13}C values observed in the spring and fall for the ELA lakes (Fig. 1) is likely related to turnover of the lake when dissolved CO_2 in the epilimnion is dominated by respired, isotopically lighter CO_2 from the hypolimnion (Rau 1980).

Northern pike additions decreased the abundance of cyprinids and changed the community composition in both lakes in this study. One year after northern pike additions, George (1994) observed significant declines in numbers of fathead minnow to 14 and 20% of original abundances in lakes 110 and 227, respectively. Species dominance shifted from fathead minnow (>80% of the volume of the community in

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Table 4. Mean concentrations (ng·g wet weight^{-1}) and range (in parentheses) of ΣHCH, ΣDDT, and ΣPCB in invertebrates from lakes 110 and 227, ELA, northwestern Ontario.

	n		ΣHCH		ΣDDT		ΣPCB	
Taxon	Lake 110	Lake 227	Lake 110	Lake 227	Lake 110	Lake 227	Lake 110	Lake 227
Zooplankton	5	7	0.48 (0.2–0.7)	0.60 (0.1–1.1)	0.6 (0.2–1.5)	0.4 (0.1–1.0)	7.4 (5.6–10.2)	7.5 (2.5–18.6)
Chaoborus spp.	2	4	0.34 (0.3–0.4)	0.18 (0.1–0.3)	1.6 (0.7–2.6)	0.4 (0.1–0.7)	24.8 (4.2–45.4)	3.7 (3.0–4.5)
Odonata	1	3	bd	0.3	0.1	0.8 (0.1–1.5)	32.8	12.5 (1.1–34.2)
Hirudinea	1	1	1.0	0.4	5.6	4.3	6.9	2.8

Note: bd, below detection.

1992) to dace (>90% in 1993) (George 1994; Elser et al. 1998). In contrast, no large changes in summer crustacean zooplankton or algal biomass (both in 1993 and 1994) or in species composition in either Lake 227 or Lake 110 were observed after northern pike were added, despite the dramatic decline in cyprinid populations (Elser et al. 1998; Sterner 1998; M. Paterson, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data; E. Schindler and D. Findlay, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data).

Prior to northern pike additions in Lake 110, fathead minnow fed upon zooplankton (Fig. 2), which is consistent with results from another ELA lake (Tallman et al. 1984). Predaceous fish introductions into Lake 110 changed the feeding behaviour of fathead minnow from a diet of pelagic invertebrates to one of benthos for the duration of the experiment (Fig. 2) and increased their relative trophic level. Although an increase in relative trophic position was also observed for northern redbelly dace from Lake 110 after northern pike additions, little change in their $\delta^{13}C$ was observed, indicating that they continued to feed upon benthic invertebrates. In Lake 227, any changes in fish diets could not be inferred from $\delta^{13}C$ and $\delta^{15}N$ because of the similarity in $\delta^{13}C$ values of the pelagic and benthic invertebrates. In this lake, there is low light penetration to the littoral zone (mean Secchi disk depth of 0.9 and 1.3 m for June–September of 1993 and 1994 as compared with 3.7 and 3.6 m, respectively, for Lake 110; E. Schindler, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data), and littoral production probably represented only a small proportion of the total primary production. The lack of discrimination in $\delta^{13}C$ between pelagic and littoral invertebrates may indicate that both groups predominantly relied upon carbon fixed by phytoplankton.

Organochlorines and Hg in food web biota

Concentrations of ΣDDT, ΣPCB, and Hg were consistently higher in cyprinids from oligotrophic Lake 110 than in cyprinids from eutrophic Lake 227. These results concur with other studies that have contrasted the concentrations of organochlorines in biota across gradients of productivity (Taylor et al. 1991; Larsson et al. 1992). Several nonexclusive explanations have been proposed for lower contaminant concentrations in fish from eutrophic lakes including differences in (*i*) contaminant loadings (Jeremiason et al. 1998), (*ii*) contaminant concentrations at the base of the food web related to particle dilution or dissolved organic carbon (Taylor et al. 1991; Larsson et al. 1992; Swackhamer and Skoglund 1993), (*iii*) among-lake differences in food chain length and bioaccumulation, and (*iv*) fish growth rates and growth dilution of contaminants (Larsson et al. 1992; Hammar et al. 1993). Some, but not all, of these explanations may account for the differences in contaminant concentrations in the fish from lakes 110 and 227.

There is little evidence that differences in organochlorine concentrations in cyprinids were the result of differences in deposition to lakes 110 and 227. Estimates of PCB deposition by Jeremiason et al. (1999) suggested that net loadings to eutrophic Lake 227 are 2.4 times higher than to oligotrophic Lake 110, primarily because of differences in watershed inputs and air–water gas exchange. These differences are in the opposite direction to trends observed for ΣPCB concentrations in cyprinids from these two lakes. Although ΣDDT loading was not quantitatively estimated for these lakes, there is no reason to expect differences for this organochlorine when compared with ΣPCB.

Evidence that concentrations of contaminants were lower at the base of the Lake 227 food web is ambiguous. Jeremiason et al. (1999) found that ΣPCB concentrations in epilimnetic seston were approximately two times higher in Lake 110 than in Lake 227. However, these differences were not observed in the dissolved fraction or on particles collected in sediment traps (Jeremiason et al. 1999). Concentrations of ΣDDT, ΣHCH, and MeHg have been measured in only one to three water samples from lakes 110 and 227. These determinations did not find strong differences in contaminant concentrations between the two lakes (D.C.G. Muir, unpublished data; P. Sellers, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data).

Organochlorines and Hg are primarily taken up by fish from their diet (e.g., Thomann and Connolly 1984), and higher prey concentrations result in higher levels in predators (Hall et al. 1997). In this study, some evidence for among-lake differences in prey organochlorine concentrations existed: higher concentrations of ΣPCB and ΣDDT were found in *Chaoborus*, and higher levels of ΣPCB were found in Odonata and Hirudinea from Lake 110. However, no significant differences in organochlorine concentrations were found for zooplankton from the two lakes. In contrast, MeHg in zooplankton was consistently higher in oligotrophic Lake 110. These data suggest that higher contaminant concentrations in fathead minnow and northern redbelly dace from Lake 110 may be due to higher pollutants in some of the invertebrates from this lake.

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Among-lake differences in organochlorine and Hg concentrations in fishes have been attributed to the length of the underlying food chain; higher concentrations of these contaminants have been found in top predators at a significantly higher trophic position, as determined by measurements of $\delta^{15}N$ (Kidd et al. 1995; 1998). Although we observed an increase in the relative trophic position of fathead minnow and pearl dace after northern pike additions to Lake 110, the concentrations of contaminants decreased over this same time period. In addition, the differences in relative trophic positions of fathead minnow in lakes 110 and 227 in the spring of 1993 are small (1.4 and 1.9‰, respectively) and do not concur with the higher contaminant concentrations in biota from the oligotrophic system. In this study, trophic position of the fathead minnow did not appear to be a significant determinant of their contaminant concentrations.

Significant relationships between concentrations of organochlorines or Hg and $\delta^{15}N$ in food web organisms have been used to quantify the trophic transfer of pollutants from primary consumers to top predators and to contrast the rate of pollutant bioaccumulation (as quantified by the organochlorine or Hg versus $\delta^{15}N$ slope) across food webs (e.g., Kidd et al. 1995). It was our intention to compare these relationships in this study to determine whether pollutants bioaccumulate to a greater or lesser extent through a food web based on high algal productivity than one based on low algal productivity. When seasonal data (1993) for zooplankton and *Chaoborus* and spring data (1993) for fathead minnow were examined, no significant relationships were found between log ΣDDT or ΣPCB and $\delta^{15}N$ in oligotrophic Lake 110 ($p = 0.33$ and 0.66, respectively, $n = 8$). Although concentrations of ΣDDT were higher in the cyprinids than in the zooplankton, the lack of a significantly relationhip was probably the result of high spring $\delta^{15}N$ values for zooplankton (Fig. 1). In contrast, ^{15}N was a significant predictor of log-transformed ΣDDT and ΣHCH but not of ΣPCB concentrations in comparable biota from Lake 227 ($p = 0.005$, $r^2 = 0.57$, $n = 12$; $p = 0.046$, $r^2 = 0.34$; and $p = 0.7$, respectively), although lipid explained more of the variability in these data ($r^2 = 0.69$ and 0.36 for ΣDDT and ΣHCH, respectively). As a result, it was not possible to determine whether organochlorines were accumulated to a greater or lesser extent through the food web of Lake 227 or Lake 110.

Lower contaminant concentrations in fishes may also be caused by faster growth rates and greater growth dilution in eutrophic lakes (Larsson et al. 1992). Fathead minnow rarely live beyond 2 years (Scott and Crossman 1973), and size frequency histograms of collections made in May–June from lakes 110 and 227 typically had two peaks that corresponded to young-of-the-year and age-1 year-classes (George 1994; M.J. Paterson, unpublished data). In 1992 (before northern pike additions), the average size of age-1 fathead minnow was greater in Lake 227 than in Lake 110, suggesting that this species had higher growth rates in the eutrophic system. Within Lake 110, mean Hg concentrations in cyprinids varied up to fivefold over the course of this study and were lowest in the fall and highest in the spring of each sampling year, presumably after periods of high and low growth, respectively (Fig. 4). This trend was not observed in Lake 227 but cannot be explained at present. Unfortunately, the size frequency distributions could not be used to interpret variations in growth rates after food web manipulations because fathead minnow sizes were then affected by both differences in growth and size selective predation by northern pike.

In conclusion, the addition of northern pike into an oligotrophic and a eutrophic lake changed the abundances and community compositions of cyprinids as expected based on existing food web theory. Stable isotope analyses revealed that fathead minnow in the oligotrophic lake shifted feeding habits from zooplankton to zoobenthos after predator introductions. However, food web manipulations did not significantly change the concentrations of organochlorines in cyprinids from either lake, despite changes in the dietary habits of one fish population and decreases in cyprinid abundances in both lakes. In part, the absence of changes in contaminant concentrations may reflect the fact that a strong trophic cascade was not observed in either lake in the first year after northern pike additions. Before and after northern pike additions, concentrations of organochlorines and Hg were higher in large invertebrates and fish from the oligotrophic lake than in those from the eutrophic lake. These among-lake differences likely arose from faster growth rates of organisms and lower concentrations of contaminants in biota at the base of the food web in the high-productivity system.

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An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*

Elena Gorokhova and Sture Hansson

Abstract: The trophic isotope fractionation of mysids, in response to a change in the isotopic composition in their diet, was examined in the laboratory. Field-caught *Mysis mixta* and *Neomysis integer* had similar $\delta^{13}C$ values, ranging between –23.1 and –21.5‰. They also had similar $\delta^{15}N$ values, and for both species, this value increased with body size. Experimental starvation did not change the isotopic composition. To test for metabolic fractionation of isotopes, the mysids were fed different diets, newly hatched *Artemia* and detrital *Enteromorpha*. The isotopic composition was monitored in abdominal muscle tissue, exoskeleton, and feces over 12 weeks. The $\delta^{13}C$ composition in muscle tissue had not reached an isotopic equilibrium with the diet at the end of the experiment, while the exoskeleton came into equilibrium with the food in 2–3 weeks. Muscle was enriched in ^{15}N relatively to the food, +3.6‰ for *Artemia* and +2.7‰ for *Enteromorpha*. The isotopic composition in muscle, exuviae, and feces may form a basis for diet reconstruction of mysids. The feces $\delta^{13}C$ and $\delta^{15}N$ values mirror the diet over the last few hours, exuviae $\delta^{13}C$ values represent nutrients metabolized 2–3 weeks ago, and muscle tissue integrates the isotopic signal over a relatively long period.

Résumé : On a étudié en laboratoire le fractionnement trophique des isotopes chez des mysidés, en réponse à un changement dans la composition isotopique de leur régime alimentaire. Des *Mysis mixta* et des *Neomysis integer* ont montré des valeurs de $\delta^{13}C$ similaires, variant entre –23,1 et –21,5‰. Ils avaient aussi des valeurs de $\delta^{15}N$ similaires, et chez les deux espèces, cette valeur augmentait avec la taille. La privation expérimentale de nourriture n'a pas changé la composition isotopique. Pour examiner le fractionnement métabolique des isotopes, on a exposé les mysidés à différents régimes alimentaires, l'un d'*Artemia* fraîchement éclose et l'autre d'*Enteromorpha* détritique. La composition isotopique a été suivie durant 12 semaines dans le tissu musculaire abdominal, l'exosquelette et les fèces. La composition isotopique de $\delta^{13}C$ dans le tissu musculaire n'a pas atteint un équilibre isotopique avec le régime alimentaire à la fin de l'expérience, tandis que l'exosquelette est parvenu à l'équilibre avec les aliments en 2–3 semaines. Les muscles étaient plus riches que la nourriture en ^{15}N, soit de +3,6‰ dans le cas d'*Artemia* et de +2,7‰ dans le cas d'*Enteromorpha*. La composition isotopique dans les muscles, les exuvies et les fèces peuvent constituer une base pour la reconstruction du régime alimentaire des mysidés. Les valeurs de $\delta^{13}C$ et de $\delta^{15}N$ dans les fèces reflètent le régime alimentaire durant les quelques dernières heures, les valeurs de $\delta^{13}C$ dans les exuvies représentent les éléments nutritifs métabolisés 2–3 semaines auparavant et le tissu musculaire intègre le signal isotopique sur une période relativement longue.

[Traduit par la Rédaction]

Introduction

In ecological research, natural abundances of stable carbon and nitrogen isotopes are being increasingly used to identify sources of organic matter and foraging location and to define trophic structures in aquatic food webs (e.g., reviews by Fry and Sherr 1984; Owens 1987). The ratio between different isotopes of carbon and nitrogen differs between organisms and their diets because of a slight selective retention of the heavy isotope and excretion of the lighter isotope. As a result, organisms tend to become enriched in heavy isotopes as compared with their food (Owens 1987). This fractionation is a continuous process whose influence on the isotopic composition of different tissues depends on their growth and turnover rates.

It is often assumed that the trophic fractionation of isotopes is relatively constant: about 3.0‰ per trophic level for nitrogen and 0.8‰ for carbon (e.g., DeNiro and Epstein 1978, 1981; Fry and Sherr 1984; Owens 1987). However, using laboratory-raised animals, DeNiro and Epstein (1981) and Macko et al. (1982) established that fractionation can be rather variable and species specific. Thus, our ability to resolve complex food web relationships requires more thorough knowledge of the isotope fractionation by the animals in question.

Despite recent advances in stable isotope analysis, few experimental studies have addressed the relationship between the isotopic composition of an animal and its food, and the

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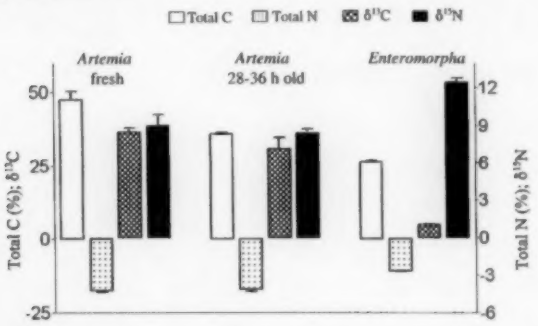
Fig. 1. Elemental and isotopic composition (mean ± SD) of the experimental diets.



response time of different tissue types to changes in the isotopic composition in the food source (Gannes et al. 1997). In muscle tissues with relatively slow turnover rates, isotope ratios reflect the diet integrated over some period (weeks to months) (Tieszen et al. 1983; Hesslein et al. 1993). In gerbils and birds, organs known to have a fast turnover (e.g., liver) and hence more rapid response than slowly growing tissues (e.g., muscle) have provided a sensitive tool when interpreting trophic interactions (Tieszen et al. 1983; Hobson and Clark 1992). The δD of bird feathers is strongly correlated with precipitation δD in the region where the feathers were grown (Hobson and Wassenaar 1997), providing information for studies of migrations. Another example is continuously growing keratinous baleen plates that reflect zooplankton isotopic composition along the migration path of whales (Schell et al. 1989). The fact that different tissues fractionate isotopes differently has important implications for the sampling and interpretation of relationships between the isotopic composition of consumers and their possible food. Hence, when using isotopes to study diets, the internal fractionation by the consumer as well as turnover rates of the analyzed tissues must be known.

For the Baltic Sea ecosystem, the food web structure as shown by stable isotope analysis follows expectations, with the notable exception of mysids (Hansson et al. 1997). In general, mysids are omnivorous predators on zooplankton and at the same time are prey for common zooplanktivore fish species (Rudstam and Hansson 1990; Rudstam et al. 1992). In laboratory feeding experiments with natural zooplankton assemblages, *Mysis mixta* was found to be a selective zooplanktivore, preferring large copepods (*Pseudocalanus* juveniles and adult *Acartia*; Mohammadian et al. 1997). The unexpectedly low $\delta^{15}N$ values for *M. mixta* observed by Hansson et al. (1997) indicate that it may be much more herbivorous than previously assumed. On the other hand, there is a possibility that *M. mixta* fractionates isotopes in nonnormal proportions, as shown, for example, for some marine amphipods (Macko et al. 1982).

Our experiments on rearing and feeding of *M. mixta* with standardized prey (Gorokhova and Hansson 1997; Gorokhova 1998) have shown that these animals are suitable for short- and long-term laboratory studies. This study examines the trophic isotope fractionation of *M. mixta* and *Neomysis integer*, which are two dominant species of Baltic mysids, in response to a change in the isotopic composition of their diet. Experimentally, we studied the rate at which muscle and exuviae equilibrate to the isotopic composition in the food. Since a difference in gastrointestinal assimilation between isotopes is the first possible step in trophic fractionation, we also analyzed fecal materials. Furthermore, diets of many species are difficult to study by means of stomach content analyses. Isotopic composition of fecal material could then give valuable diet information provided that gastrointestinal fractionation is known. Experiments were also carried out to study the effect of starvation on mysid isotopic composition.

Materials and methods

Field sampling

Specimens of *M. mixta* and *N. integer* were collected in a coastal Baltic Sea area (58°50′N, 17°33′E) in August 1996 and June 1997. Immediately after the collection, the body lengths (millimetres) of 41 individual mysids were measured. Measurements were taken from the tip of the rostrum to the end of the last abdominal segment using calipers (± 0.02 mm) under a stereomicroscope. Thereafter, animals were prepared for analyses of elemental composition and isotope ratios. Simultaneously, materials were collected for the development of regression models between various body variables, such as uropod length, total body length (millimetres), wet weight (milligrams), and dry weight (milligrams).

Long-term feeding experiments

These experiments were carried out during August–November 1996 in a laboratory at the Department of Systems Ecology, Stockholm University. Except when we worked with them, the mysids were kept in darkness and at 9–10°C, i.e., conditions similar to those in their natural environment. Until the experiments started, they were fed with newly hatched nauplii of *Artemia* spp.

During the experiments, the animals were kept individually in 1-L plastic beakers filled with filtered seawater (6–7‰) and offered excess amounts of *Artemia* spp. nauplii or detrital *Enteromorpha* spp. The *Artemia* nauplii (San Francisco Bay brand) were hatched in a 2-L container supplied with aeration (23–26‰, 26–28°C) and fed to the mysids within 36 h of hatching. The detritus was made from air-dried plants collected in a rock pool, which were crumbled, suspended in seawater, and allowed to decompose at room temperature for at least 1 week.

Samples of food were collected for isotope analyses during the course of the experiments. The age of the *Artemia* offered to the mysids ranged between newly hatched (less than 3 h) and 28–36 h old. The possibility that metabolic losses caused isotope ratio differences between nauplii of different ages was tested. Five replicates for each group were analyzed separately. Nauplii of both ages were isolated from shells, rinsed with deionized water, and filtered onto 250-μm screens for drying. Detrital *Enteromorpha* was also sampled on five occasions. Figure 1 summarizes the dietary conditions to which the animals were subjected during the experiments.

The initial isotopic composition of the animals used in the experiments was assumed to be that of mysids from the same size group (± 0.2 mm) sampled at the start of the experiments.

Experiment I. *Artemia* diet

Forty-five juvenile specimens of *M. mixta* (length 8–11 mm) were fed ad libitum on an *Artemia* diet. The food was changed daily, at which time, each container was checked for the exuvia remains. When moulting had occurred, the moult was removed and the mysid was transferred to a transparent plastic tube for length measurement and then returned to the experimental container. The growth at moult was estimated as moult size increment in length

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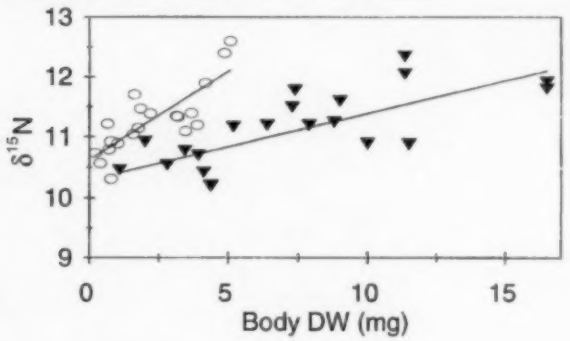
Table 1. Arithmetic means ± SD for body dry weight (DW), total carbon, and total nitrogen of *M. mixta* and *N. integer* in the field samples and biometric equations for the relationships between total body length (*L*, mm), uropod length (*U*, mm), and DW (mg) for the pooled field and laboratory data.

	M. mixta	*N. integer*
DW (mg)	2.74±1.25 (21)	1.67±0.32 (20)
Total carbon (%)	42.80±2.69 (21)	41.69±2.19 (20)
Total nitrogen (%)	10.97±0.64 (21)	11.11±0.23 (20)
L–DW	$DW = 0.00317 \times L^{2.85}$ (46), $r^2 = 0.89$, $P < 0.05$	$DW = 0.0041 \times L^{2.78}$ (51), $r^2 = 0.97$, $P < 0.0001$
U–*L*	$L = 3.60 \times U + 1.40$ (26), $r^2 = 0.98$, $P < 0.0001$	$L = 4.59 \times U + 1.55$ (15), $r^2 = 0.87$, $P < 0.05$

Note: Number of individuals analyzed given in parentheses.

Fig. 2. Variations in $\delta^{15}N$ values with body size (dry weight) of *M. mixta* (▼) and *N. integer* (○) in wild populations.



(millimetres) and converted to dry weight (milligrams) using the biometrical equations in Table 1.

Every week, two ot three individuals were sacrificed, rinsed briefly with deionized water, and transferred to plastic capsules for drying in preparation for stable isotope analysis. Cast exuviae were treated the same way. Five pooled samples of feces were collected during the experiment at approximately equal sampling intervals. The experiment lasted for six to eight moult cycles (12–13 weeks).

Experiment II. *Enteromorpha* diet

Five specimens of *M. mixta* were fed on a diet of *Enteromorpha* and incubated for the same time and under similar experimental conditions as those fed *Artemia* (experiment I). The food was changed twice every week, but since exuviae could not be properly cleaned from detritus, they were not sampled. The initial size and final size of each mysid were measured as described above and the entire animals were sampled for isotope analyses at the end of the experiment.

Experiment III. Short-term moulting and gut evacuation experiment

This experiment was designed to allow the direct determination of exuviae and feces isotopic composition in wild mysids. Within 2 h of collection in July 1997, *M. mixta* and *N. integer* were sorted individually into 100-mL plastic jars, each also holding a natural mixture of zooplankton at ambient concentrations (50–80 individuals·L^{-1}). The jars were incubated in darkness (5–8°C) for 48 h but taken out at 3-h intervals for visual checks. When a mysid had moulted, the exuvia was removed and prepared for isotopic analysis. At every check, feces for isotopic analysis were collected from randomly chosen jars and pooled together by the time of sampling but separately for the two mysid species.

Experiment IV. Starvation experiment

To obtain data on the effect of starvation on mysid isotopic composition, 30 subadults of *N. integer* were starved for 5 weeks at 10°C during October–November 1996. By this time, mysids had been in captivity for more than 2 months. Specimens were placed individually in 500-mL plastic jars, and to prevent them from consuming their cast exuviae, the jars were inspected daily. At weekly intervals, three individuals were sacrificed, dried, and weighed in preparation for isotopic analysis.

Sample preparation and isotopic analysis

Mysids and exuviae were placed in 5-mL vials and dried at 60°C to constant weight. Dry weights were determined using a Sartorius M3P microbalance (± 0.001 mg). When mysid abdominal tissue was analyzed, abdomens were carefully separated while dried. Dry material was ground in an agate mortar. Whole shrimp/abdomen or its aliquot, depending on size, or whole exuvia comprised each sample. Fecal pellets were pipetted off the experimental container bottom with a wide-bore pipette and transferred to 5-mL vials containing deionized water and left for a few minutes to minimize addition of dissolved matter. To avoid contamination with food remains, a stereomicroscope and a Pasteur pipette were used to transfer individual pellets from the vial directly to preweighed tin capsules. These samples were dried overnight at 60°C. Samples of food items were dried the same way, ground with mortar, and weighed into tin capsules. All samples were kept frozen until analyzed.

The C:N ratio and the $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios in whole mysids, abdominal muscle tissue, exuviae, feces, and food items were analyzed on a Carlo Erba elemental analyzer (E1108 CHNS-O) connected to a Fison Optima continuous-flow mass spectrometer. Three working standards (pike muscle) were run for every 15 samples and analytical and method blanks were included. The standard deviation among replicate standard samples was within 0.2‰ for both carbon and nitrogen. If not stated otherwise, data are given as means and standard deviations.

Results

$\delta^{13}C$ and $\delta^{15}N$ variations in field samples

In August 1996, most of the mysids caught were subadult *N. integer*, while juveniles of *M. mixta* dominated catches in July 1997. Overall, $\delta^{13}C$ and $\delta^{15}N$ values of the two species were similar and ranged from –23.1 to –21.5‰ for $\delta^{13}C$ and from 10.2 to 12.6‰ for $\delta^{15}N$ (Fig. 2; Table 2). Neither *N. integer* nor *M. mixta* showed significant differences in $\delta^{13}C$, $\delta^{15}N$, and C:N ratios between the years of sampling or between sexes (Mann–Whitney test, $P > 0.05$ in all cases), and samples were pooled for regression analyses. The $\delta^{15}N$ values increased with size for both *M. mixta* ($\delta^{15}N = 0.11 \times$ body dry weight + 10.20, $n = 21$, $r^2 = 0.55$) and *N. integer*

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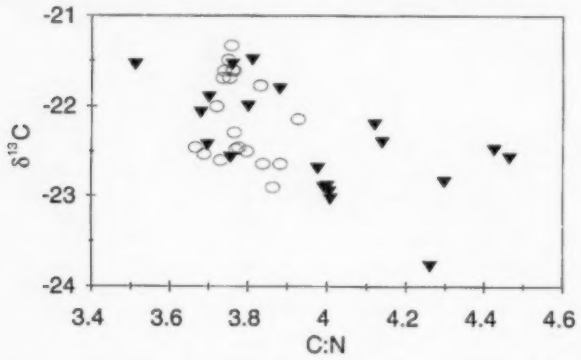
Table 2. Isotopic composition of mysid body, muscle tissue, exuviae, and feces from laboratory and field samples.

	$\delta^{13}C$		$\delta^{15}N$	
	Laboratory	Field	Laboratory	Field
M. mixta				
Whole body		−22.32±0.55 (21)		10.97±0.75 (20)
Muscle	−19.90±0.34 (5)	−22.61±0.48 (16)	12.21±0.88 (5)	10.61±0.39 (16)
Exuviae	−18.26±0.99 (5)	−21.20±0.30 (16)	6.74±1.22 (5)	5.02±0.21 (16)
Feces	−15.96±1.02 (5)	−20.61±0.24 (5)	12.13±1.07 (5)	10.72±0.22 (5)
N. integer				
Whole body		−22.30±0.44 (20)		11.07±0.36 (20)
Muscle	−19.28±0.22 (3)	−22.12±0.31 (6)	14.72±0.18 (3)	11.08±0.38 6)
Exuviae		−21.04±0.23 (6)		4.92±0.28 (6)
Feces		−20.76±0.28 (3)		11.16±0.22 (3)

Note: Number of samples analyzed given in parentheses. Laboratory data for *M. mixta* muscle tissue and exuviae are final values in experiment I and for *N. integer* are initial values in experiment IV.

Fig. 3. Effect of the lipid content (expressed as the C:N ratio) on $\delta^{13}C$ of *M. mixta* (▼) and *N. integer* (○) in wild populations.



($\delta^{15}N = 0.29 \times$ body dry weight + 10.63, $n = 20$, $r^2 = 0.65$) (Fig. 2), but with a significant difference between the species (homogeneity of slopes test, $F = 32.9$, $P < 0.0001$).

While we found no significant correlation between size/age and the $\delta^{13}C$ value, the carbon isotopic composition of *M. mixta* was weakly but significantly correlated with the C:N ratio ($\delta^{13}C = -1.41 \times$ C:N ratio − 16.8, $n = 21$, $r^2 = 0.41$, $F = 17$, $P < 0.0002$) (Fig. 3). The C:N ratio also differed significantly between mysid species (unpaired t test, $P < 0.01$; F test, $P < 0.0001$), with *M. mixta* having both higher variability and higher absolute values compared with *N. integer* (3.94 ± 0.27 and 3.78 ± 0.06, respectively).

Diet-dependent isotope fractionation (experiments I and II)

The two types of food supplied to the mysids in the laboratory had significantly different elemental and isotopic composition (Fig. 1; Table 3). The comparison between newly hatched and 28- to 36-h-old *Artemia* did not indicate differences in $\delta^{13}C$ and $\delta^{15}N$ values (Mann–Whitney test, $P > 0.7$), and we have used the averages (Table 3) in the analyses below. In spite of the difference in elemental composition between food types, both diets fulfilled the nutritional requirements of the laboratory-cultured *M. mixta*. A comparison of the percentage gain in dry weight of mysids fed *Artemia* and *Enteromorpha* for 12 weeks showed no significant difference between treatments (122 ± 60 and 110 ± 55%, respectively; unpaired t test, $P > 0.4$). These values match previous estimates of growth rates from field populations (Rudstam 1989).

Table 3. $\delta^{13}C$ and $\delta^{15}N$ of *M. mixta* at the beginning and end of each experiment and of their food sources (*Artemia* and *Enteromorpha*) in 12-week feeding experiments.

	$\delta^{13}C$	$\Delta^{13}C$	$\delta^{15}N$	$\Delta^{15}N$
Experiment I		−2.59		3.55
Artemia	−17.31±0.65		8.65±0.80	
M. mixta, beginning	−22.52±0.48		10.77±0.41	
M. mixta, end	−19.90±0.34		12.21±0.88	
Experiment II		−7.44		2.69
Enteromorpha	−10.95±0.12		12.40±0.34	
M. mixta, beginning	−22.61±0.39		10.82±0.52	
M. mixta, end	−18.39±0.43		15.09±0.43	

At the beginning of experiments I and II, $\delta^{13}C$ and $\delta^{15}N$ values were virtually identical between treatments (Table 3), but in both experiments the 12-week incubation with standardized food resulted in measurable changes in isotopic composition. On both diets, the isotope ratios in the abdominal muscle of *M. mixta* increased. Relative to the diet, mysids were substantially depleted in heavy isotopes of carbon (when fed *Artemia* the difference between mysid and food was −2.6‰ and on an *Enteromorpha* diet was −7.4‰). The difference in $\delta^{15}N$ between the mysids and *Artemia* and *Enteromorpha* was +3.6 and +2.7‰, respectively.

Isotopic response of mysid muscle and exuvia to an *Artemia* diet (experiment I)

The moult cycle duration of the mysids was slightly prolonged with increasing size/age and was on average 12.6 days. Each successive moult resulted in an average increase of 9.2% in body length and of about 30% in the preecdysial dry weight (estimated from length–weight relationships). There was no significant difference in the moult increment between sexes or between the laboratory-raised and field-caught animals (see experiment III; two-way ANOVA, df = 1,108: sex, $P > 0.4$; origin (laboratory or field), $P < 0.3$; interaction, $P > 0.6$).

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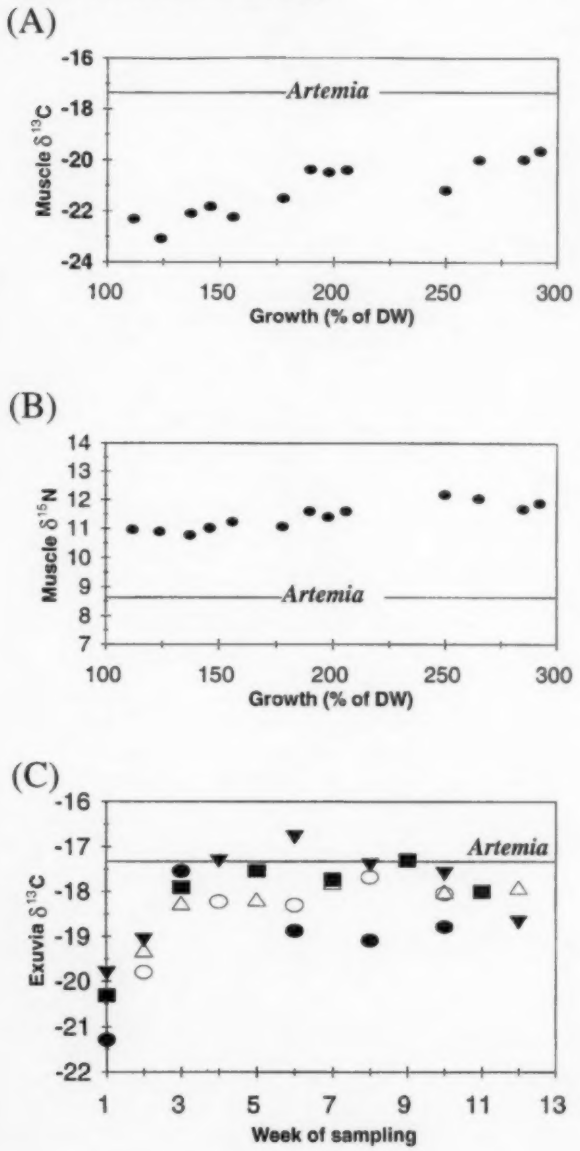
Fig. 4. Temporal changes of isotopic composition in *M. mixta* in response to the experimental diet. The isotopic composition of the diet is shown as the *Artemia* line. (A) Muscle tissue $\delta^{13}C$ values (each data point represents a pooled sample of two or three individuals); (B) muscle tissue $\delta^{15}N$ values (each data point represents a pooled sample of two or three individuals); (C) exoskeleton $\delta^{13}C$ values (symbol of a given type represents exuviae shed by the same individual).



There was a general shift in muscle tissue $\delta^{13}C$ towards the composition in the diet; however, it appeared not to have reached isotopic equilibrium within the 12 weeks of the experiment (Fig. 4A). This was, however, the case for muscle $\delta^{15}N$, which seems to have reached equilibrium in 8–9 weeks from the beginning of the experiment, when mysid weight had increased by a factor of 2 or more (Fig. 4B).

The mysid exoskeleton approached isotopic equilibrium much faster than the muscle. In 2–3 weeks, $\delta^{13}C$ values of exuviae approached that of the diet and reached apparent equilibrium (Fig. 4C). Exuviae collected from the second moulting period and later did not differ significantly from *Artemia* (–18.26 ± 0.997 and –17.31 ± 0.65‰, respectively; unpaired t test, df = 16, $P > 0.2$). The nitrogen isotope ratios in the exuviae did not show any consistent trend but were depleted (–2.4‰ on average) relative to the diet (unpaired t test, df = 22, $P < 0.001$).

Internal metabolic fractionation (experiments I and III)

The isotopic composition of carbon and nitrogen differed between products of mysid metabolic activity (muscle tissue, exuviae, and feces) (Table 2). By the end of experiments I and III, the following internal fractionation for both laboratory-maintained animals was obtained: $\delta^{13}C_{feces} > \delta^{13}C_{exuviae} > \delta^{13}C_{muscle}$ and $\delta^{15}N_{feces} \approx \delta^{15}N_{muscle} > \delta^{15}N_{exuviae}$. Field observations matched this trend (Table 2).

Two-way ANOVA statistics were used to test for differences in the isotope ratios between newly caught and laboratory-grown *M. mixta* and between muscle tissue and feces or exuviae (cf. Table 2). To balance the analyses, we randomly drew five samples from the 16 samples of muscle and exuviae from the data on newly caught animals.

The carbon isotopic composition differed significantly between the two groups of mysids ($P < 0.001$), between muscle and feces ($P < 0.001$; higher $\delta^{13}C$ values in feces), and between muscle and exuviae ($P < 0.001$; higher $\delta^{13}C$ values in exuviae). There was also a significant interaction ($P < 0.001$) caused by a larger difference between muscle and feces in samples from laboratory-held mysids (+3.9‰) than in samples from newly caught animals (+1.7‰). The average difference between $\delta^{13}C$ in exuviae and muscle tissue was 1.42‰ and did not differ between newly caught and laboratory-reared mysids.

Samples of muscle, feces, and exuviae of laboratory-reared mysids had generally higher $\delta^{15}N$ values than samples from newly caught specimens ($P < 0.002$). Muscle had significantly higher $\delta^{15}N$ values ($P < 0.001$; difference in $\delta^{15}N$ = +5.5‰) than exuviae without a difference between newly caught and laboratory-reared mysids ($P > 0.1$). No difference between muscle and feces was found ($P > 0.5$). The degree of internal fractionation was not species specific when comparing $\delta^{15}N$ among muscle and exuviae. Exuviae in both *M. mixta* and *N. integer* were enriched in $\delta^{13}C$ (mean ±SE = +1.08 ± 0.16 and +1.41 ± 0.14‰, respectively) and depleted in $\delta^{15}N$ (–6.16 ± 0.19 and –5.59 ± 0.11‰, respectively) relatively to muscle (experiment III; unpaired t test, $P < 0.0001$).

Higher variations among both exuvia and feces samples in experiment I compared with those in experiment III were most probably caused by longer sampling intervals and higher temperature in experiment I, resulting in higher decomposition rates.

Effect of starvation on muscle tissue isotopic composition (experiment IV)

A pilot experiment showed that juvenile and subadult *N. integer* survived no longer than 7 weeks when starved at 9°C, and our starvation experiment was limited to 5 weeks. After this period, the spontaneous mortality had reached

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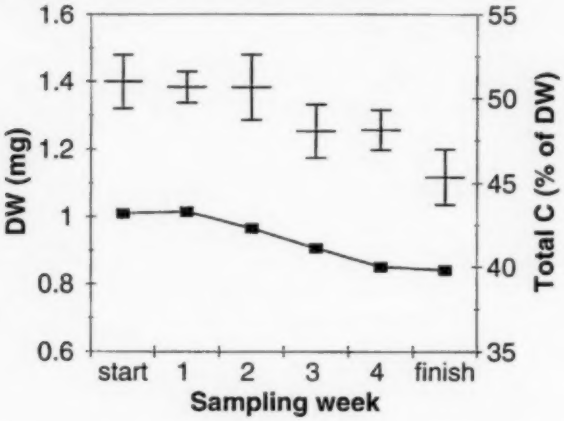
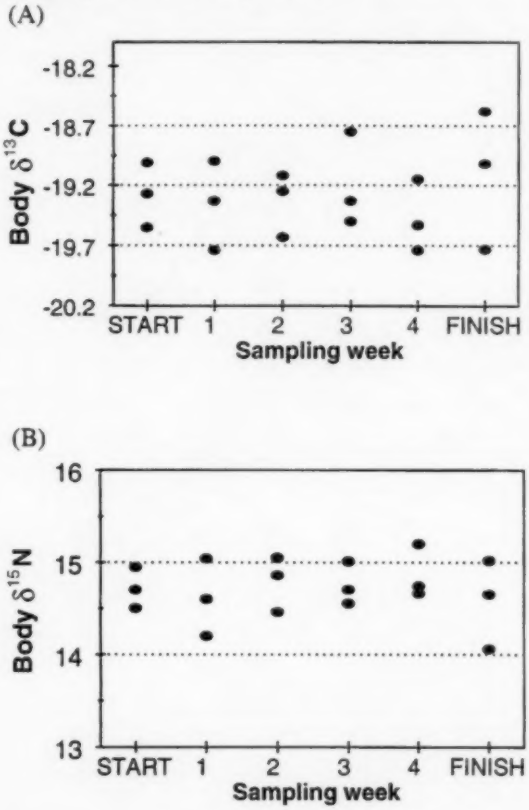
Fig. 5. Changes in *N. integer* dry weight (horizontal line represents the mean value and vertical bars represent the standard deviation) and total carbon content (■) as a percentage of dry weight during the starvation experiment.



36% and the average dry weight decreased by 18.6%. The overall reduction of the total body carbon was 7.6%, indicating a substantial loss of organic reserves (Fig. 5). Neither $\delta^{13}C$ nor $\delta^{15}N$ exhibited a significant change over the starvation period (Fig. 6).

Fig. 6. Variations in *N. integer* body (A) $\delta^{13}C$ and (B) $\delta^{15}N$ values during the starvation experiment.



Discussion

The potential for omnivory in mysids implies that they may modify their diets in response to changes in food quality and abundance, as predicted by optimal foraging theory (Hughes 1980). This gives a possible explanation for the somewhat contradictory data published on the isotopic composition of mysids in the northern Baltic proper. Rolff et al. (1993) found that the $\delta^{15}N$ values for *M. mixta* were too high for their assumed zooplanktivory (6.2‰ for zooplankton and 10.9‰ for mysids). Data on $\delta^{13}C$ in this study was "unclear and does not appear to give reliable trophic information." On the contrary, Hansson et al. (1997) reported that the same mysid species had consistently lower $\delta^{15}N$ (about 8‰) than expected from zooplanktivorous feeding; the difference between zooplankton and mysids was only 0.4‰. Again, $\delta^{13}C$ values were "less consistent" and difficult to interpret.

The average $\delta^{15}N$ value of wild-caught mysids (about 11‰ for both species) was close to those reported by Rolff et al. (1993) and Rolff (1998) for *M. mixta* and higher than the value obtained by Hansson et al. (1997). Due to seasonal changes in isotopic composition of both mysids and their potential food sources, only data for August were compared. The $\delta^{13}C$ isotope ratios of mysids in our study were rather homogeneous (mean = –22.3‰ for both *M. mixta* and *N. integer*) (Table 2) and lower than those reported previously by Rolff (1998) (–21.2‰ for *M. mixta* and –19.4‰ for *N. integer*). According to the values reported by Rolff (1998) for zooplankton in August 1994, $\delta^{13}C$ and $\delta^{15}N$ of adult copepods were –22.4 and 6.7‰, respectively, but –21.7 and 3.0‰ for the rest of the mesozooplankton. This suggests that both mysid species may be feeding on the largest mesozooplankton, namely older copepodites and adults (cf. Mohammadian et al. 1997). However, isotopic values for cyanobacteria ($\delta^{13}C$ = –22.9‰ and $\delta^{15}N$ = 4.5‰; Rolff 1998) and for microbially degraded material in the oxygenated water column ($\delta^{15}N$ = 8‰; Voss et al. 1997) do not rule out detritus and particulate organic matter as a possible food sources. Furthermore, the survival and growth of *M. mixta* fed detrital *Enteromorpha* in our experiments show that this type of food can meet its nutritional requirements.

The laboratory feeding experiments were designed to estimate trophic enrichment factors for carbon and nitrogen isotopes. At the beginning of the experiments, $\delta^{13}C$ and $\delta^{15}N$ values of *M. mixta* muscle (–22.6 and 10.8‰, respectively) differed by +11.6 and –1.6‰ and –5.2 and +2.1‰ from the values of detrital *Enteromorpha* and *Artemia*, respectively. By the end of the experiments, the $\delta^{13}C$ and $\delta^{15}N$ values had changed to –18.4 and 15.1‰ and –19.9 and 12.2‰ for mysids fed *Enteromorpha* and *Artemia*, respectively. Assuming that the nitrogen isotopic composition had reached equilibrium with the diet, this corresponds to the $\delta^{15}N$ trophic fractionation of 2.7 and 3.6‰ for the two diets. Thus, regardless of great differences in isotopic signatures of two food sources, the $\delta^{15}N$ enrichment factors were still comparable. This finding is close to the results of DeNiro and Epstein (1981) and Macko et al. (1982) who found the $\delta^{15}N$ values of a species fed different diets to be quite similar. Our result can be compared with the $\delta^{15}N$ enrichment of

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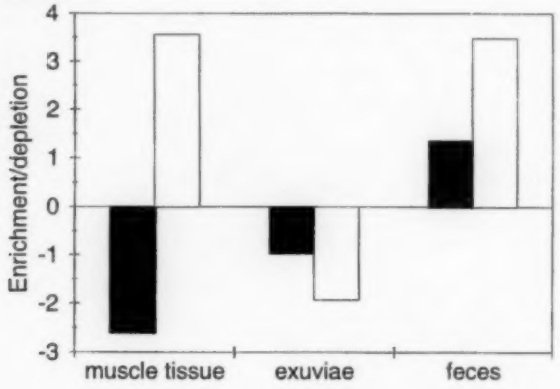
Fig. 7. Experimental data on trophic fractionation for ^{13}C (open bars) and ^{15}N (solid bars) in *M. mixta* by muscle tissue, exuviae, and feces.



+3.6‰ for the large predatory invertebrates (euphausids and pandalids) on Georges Bank (Fry 1988) and the nitrogen enrichment of +3.2‰ in laboratory-reared *Neomysis intermedia* (Toda and Wada 1990).

Ontogenetic changes in the isotopic composition can be caused either by decreased growth efficiency with increasing size (Minagawa and Wada 1984; Gorokhova 1998) or by a diet shift to food from higher trophic levels. For $\delta^{15}N$, such ontogenetic changes were recorded for both *M. mixta* and *N. integer* (Fig. 2). For $\delta^{13}C$, other factors can also influence the isotopic composition. Individuals that are rich in lipids (a high C:N ratio) can be expected to have low $\delta^{13}C$ values (Fry and Sherr 1984), and we found that *M. mixta* $\delta^{13}C$ values reflected their lipid content expressed as the C:N ratio. Since the lipid content in mysids varies seasonally (Adare and Lasenby 1994), proper interpretation of isotope data in relation to food web structure needs lipid corrections for $\delta^{13}C$ values, size corrections for $\delta^{15}N$, and size-specific sample preparation for stable isotope analysis.

It has been suggested that starving animals should show a progressive increase in the heavy isotope concentration, since mainly light isotopes are used in catabolism, and at starvation, these are not replaced (Hobson et al. 1993; Gannes et al. 1997). However, we found no significant changes in any of the isotopes studied. These findings are similar to those of Frazer et al. (1997) who reported insignificant changes in larval krill during 8 weeks of starvation. Therefore, these results do not support the assumption that catabolic processes lead to the isotopic variation in starving mysids.

Even though in our experiments, animals were fed isotopically invariable and homogeneous food for 12 weeks, the carbon isotopic composition in muscle tissue did not reach the $\delta^{13}C$ value of the food. The observed growth rates predict that a significant portion of the ingested carbon was used for somatic growth, with all newly added biomass having the isotopic signature reflecting that of the experimental food. The slow approach by muscle tissue (assumed to be mostly proteins) $\delta^{13}C$ towards the diet composition may be caused by (*i*) differential assimilation of carbon from amino acids, lipids, carbohydrates, etc., or (*ii*) differential allocation of assimilated carbon among tissues. Our data on isotope signature dynamics of exuviae in response to the experimental diet support the latter explanation. Because exoskeleton is composed of nutrients that are derived from the diet, the relative abundances of carbon and nitrogen isotopes in exuviae should be related to those in food. Indeed, in our experiments, exuviae were consistently depleted in ^{15}N, but their $\delta^{13}C$ values did not differ from those of the diet.

Development of the new exoskeleton occurs during the last moulting stages (Drach 1939) and involves enzymatic digestion of the existing postecdysial layer and reabsorption of material from this layer into the new cuticle (Lockwood 1968). At the time of moult (ecdysis), this layer is fully developed, and we refer to it as "preecdysial." Another portion of the exoskeleton is formed after ecdysis, and the synthesis of this postecdysial layer is associated with incorporation of assimilated nutrients into newly formed matter. Thus, the old cuticle (exuvia) contains material synthesized during one moult cycle before the last one. An obvious advantage in using cast exuviae for diet reconstruction is that their $\delta^{13}C$ value appears to be in a good balance with the carbon isotopic composition in the diet during the time of exoskeleton secretion.

Digestion leads to increased $\delta^{13}C$ and $\delta^{15}N$ values in the feces compared with the diet (+1.4 and +3.4‰, respectively) (Fig. 7). For nitrogen, the fractionation is practically the same as for the muscle tissue.

In conclusion, the isotopic composition in muscles, exuviae, and feces may form a basis for diet reconstruction of arthropods like mysids. The feces $\delta^{13}C$ and $\delta^{15}N$ values mirror the diet over the last few hours, exuviae $\delta^{13}C$ values represent nutrients metabolized 2–3 weeks ago, and muscle tissues integrates the isotopic signal over a relatively long period (6–8 weeks for $\delta^{15}N$ and more than 3 months for $\delta^{13}C$). This sampling approach might also be applicable to other crustaceans with a soft, little-mineralized exoskeleton. Likewise, Hesslein et al. (1993) suggested the potential usefulness of combined measurements in different tissues (liver and muscle) when studying the isotopic composition of fish in relation to their food source. To determine which specific tissues should be analyzed for a particular consumer, investigations of the fate of assimilated nutrients within the animal are needed. Provided that fractionation factors for different tissues are measured under controlled conditions, the use of this strategy offers the advantages of both sensitivity and specificity for studies on dietary reconstruction and food web interactions.

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Biogeochemical influences on the carbon isotope signatures of Lake Ontario biota

M.F. Leggett, M.R. Servos, R. Hesslein, O. Johannsson, E.S. Millard, and D.G. Dixon

Abstract: Particulate organic matter (POM), zooplankton, and benthic invertebrates were sampled seasonally at a midlake and east basin site in Lake Ontario. The $\delta^{13}C$ values of POM samples were compared with measurements of chlorophyll *a* concentrations, areal rates of primary productivity, concentrations of dissolved free CO_2 ($CO_{2(aq)}$), and the $\delta^{13}C$ of zooplankton and benthic invertebrates. The $\delta^{13}C$ of POM was significantly correlated with the concentration of $CO_{2(aq)}$ at the east basin site. No correlation was found between chlorophyll *a* concentrations, areal rates of primary productivity, and the $\delta^{13}C$ of POM. The $\delta^{13}C$ of zooplankton was not always similar to the $\delta^{13}C$ of the POM fraction collected, indicative of the complexity of food web interactions within the lower trophic levels. The $\delta^{13}C$ of benthic invertebrates feeding on the sediment surface was similar to that of POM produced prior to stratification, indicating the importance of the spring bloom as a food source for benthic primary consumers.

Résumé : La matière organique particulaire, le zooplancton et les invertébrés benthiques ont fait l'objet de prélèvements saisonniers dans la partie centrale et le bassin est du lac Ontario. Les $\delta^{13}C$ des échantillons de matières organiques particulaires (MOP) ont été comparés aux concentrations de chlorophylle *a*, aux taux surfaciques de la productivité primaire, aux concentrations de $CO_{2(aq)}$ et aux $\delta^{13}C$ du zooplancton et des invertébrés benthiques. Le $\delta^{13}C$ des MOP présentait une corrélation significative avec la concentration de $CO_{2(aq)}$ au site du bassin est. Aucune corrélation n'a été décelée entre les concentrations de chlorophylle *a*, les taux surfaciques de la productivité primaire et le $\delta^{13}C$ des MOP. Le $\delta^{13}C$ du zooplancton n'était pas toujours semblable à celui de la fraction des MOP prélevées, ce qui dénote la complexité des interactions du réseau alimentaire des niveaux trophiques inférieurs. Le $\delta^{13}C$ des invertébrés benthiques s'alimentant à la surface des sédiments était semblable à celui des MOP produites avant la stratification, ce qui montre l'importance de la prolifération printanière comme source de nourriture pour les consommateurs primaires benthiques.

[Traduit par la Rédaction]

Introduction

Carbon isotopes have been used effectively as tracers of food sources in aquatic systems. It has also been suggested that the $\delta^{13}C$ of sediment and particulates may be used as an indicator of lacustrine trophic status (Gu et al. 1996). Temporal changes in the productivity of a lake have been inferred in paleolimnological investigations from the $\delta^{13}C$ of organic carbon and calcite in sediment cores (McKenzie 1985; Schelske and Hodell 1991; Hodell and Schelske 1998). The basis for these inferences is that the ^{12}C isotope of carbon is used preferentially by algal cells in photosynthesis, leaving a dissolved inorganic carbon (DIC) pool enriched in ^{13}C. Therefore, higher productivity is reflected in elevated $\delta^{13}C$ in calcite and organic carbon produced in the epilimnion (Schelske and Hodell 1991). It is assumed that the uptake of carbon by primary producers is the predominant influence on the $\delta^{13}C$ of DIC and therefore the organic carbon produced using DIC as a substrate for photosynthesis. A seasonal fluctuation in the $\delta^{13}C$ of DIC and organic carbon, reflecting seasonal changes in the magnitude of primary production, is predicted. A linear relationship between the concentration of chlorophyll *a* (Chl *a*) and (or) the rate of productivity and the $\delta^{13}C$ of primary producers might also be expected.

The amount and composition of available DIC vary between systems and are influenced by biological processes of respiration and photosynthesis as well as equilibrium kinetics and atmospheric gas exchange (Herczeg and Fairbanks 1987; Aravena et al. 1992). The relative magnitudes of the forms of DIC and the processes influencing the equilibrium between components of the lake carbonate system influence the carbon isotope signatures of particulate organic matter (POM) in two ways. First, the components of the lake carbonate system, seeking to attain a state of equilibrium chemically, also move toward a state of isotopic equilibrium

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(Weiler 1974). Many of the processes influencing the composition of the DIC pool also serve to alter the $\delta^{13}C$ of dissolved free CO_2 ($CO_{2(aq)}$) and bicarbonate (HCO_3^-). The $\delta^{13}C$ of fixed carbon will be determined to some degree by the $\delta^{13}C$ of the inorganic carbon used in photosynthesis (Rau et al. 1989; Fogel et al. 1992). Second, the observed level of isotope fractionation in primary producers is a function of the amount of carbon available at the site of activity of the enzyme binding the carbon substrate in carbon fixation (Farquhar et al. 1982; Fogel and Cifuentes 1993). Isotope fractionation is also dependent on the level of isotopic discrimination characteristic of the particular enzyme system used by the cell in carbon fixation (Goericke et al. 1994).

There may be species-specific differences in the $\delta^{13}C$ of primary producers related to differences in DIC source and carbon uptake kinetics. Because the isotope signature is a function of the physical–chemical environment and intracellular carbon concentrations, differences in cell size and morphology, or adaptation to different environments, are likely to be expressed as variation in the $\delta^{13}C$ of different species. This variation would overlay a seasonal shift of varying magnitude depending on the system observed. The zooplankton and benthic consumers feeding on primary production will have a $\delta^{13}C$ that reflects their food source. Temporal changes in the carbon signature of primary producers should be reflected in the signatures of these primary consumers. It is therefore important to characterize the baseline signature of the system and the range of signatures in available food sources in order to effectively use isotope data to make inferences on the relative trophic position of different organisms.

Given the number of areas where establishing a baseline has relevance in the interpretation of isotope data, there are relatively few studies where the isotope signature of POM is measured on a seasonal basis or in relation to system productivity (e.g., McCabe 1985; Bernasconi et al. 1997). In this study, we examined the relationship between seasonal concentrations of Chl *a*, the level of photosynthetic production, and $\delta^{13}C$ of POM to determine if the carbon isotope signature of POM could be directly correlated with the productivity of the system. Seasonal trends in the $\delta^{13}C$ of zooplankton and benthic invertebrates were measured to determine the extent to which the signature of POM was reflected by primary consumers.

Materials and methods

Two sampling sites in Lake Ontario of the Laurentian Great Lakes were chosen for comparison and a 2-year seasonal study was conducted. One site was located midlake, Station 41 (43°43′18″N, 78°01′01″N), where the water column has a depth of about 130 m. The other site was in the eastern basin, Station 81 (44°01′00″N, 76°40′18″N), where the water column is about 34 m deep (Johannsson et al. 1998). Terrestrial inputs to the POM were expected to be negligible at both sites. Lake Ontario is a mesotrophic lake with an average pH of 7.8–8.6 and high ambient concentrations of DIC (about 1800 μM). The lake stratifies in mid-June and biologically induced precipitation of $CaCO_3$ occurs seasonally at the end of July or early August (Lean et al. 1987; Hodell et al. 1998). All of the sampling for this project was done in conjunction with the Lake Ontario Bioindex biomonitoring program (Johannsson et al. 1998). Samples were collected at Stations 41 and 81 every 2 weeks from April 20 to September 27, 1994, and then again from May 11 to October 31, 1995.

Water samples for nutrient analysis and determination of both the $\delta^{13}C$ of DIC and the $CO_{2(aq)}$ concentration were obtained from discrete 10-L water samples collected with Nisken bottles. Samples were taken at 10 m when the lake was thermally homogenous and from the midepilimnion once the lake had stratified. Subsamples of water from the Nisken bottles were collected in evacuated 250-mL bottles containing KCl as a preservative for carbon isotope and $CO_{2(aq)}$ analyses. Extraction and analyses of these samples was conducted as per standard methods at the Department of Fisheries and Oceans, Freshwater Research Institute. On August 2, 1995, duplicate discrete samples were collected hourly from 3 m depth between 6:00 and 22:00 to determine if there was any diurnal fluctuation in the $\delta^{13}C$ of DIC. DIC samples were also collected on August 2, 1995, at depths of 7.5, 12.5, 25.0, 75.0, and 120.0 m; the mixing depth on August 2 was 6.5 m.

Samples of POM were obtained from 200 L of water pumped from the midepilimnion through a 44-μm Nitex screen into storage containers. Most of these samples were then sequentially filtered through 20-μm-mesh Nitex screen and precombusted (500°C) 0.7-μm nominal pore size Whatman GF/F glass fibre filters. The exception was the sample collected on a GF/F filter on May 10, 1995, at Station 81, which was not prefiltered through 20-μm-mesh Nitex screen. Filters and POM collected on 20-μm-mesh Nitex screens (when available in sufficient mass) were dried at 60°C prior to analysis. In May and June of 1995, at both sites, additional samples were obtained using a Westfalia continuous-flow centrifuge to separate POM from 200-L samples, collected as described above. POM obtained from the centrifuge was dried at 60°C prior to analysis.

Zooplankton samples were collected from the surface to 1 m above the start of the thermocline in vertical tows using 64-μm-mesh Nitex nets. Samples were screened to separate zooplankton into size categories >110, >210, and >250 μm. Screened samples were preserved in ethanol to facilitate speciation and removal of particulate and detrital material. Samples of individual species were obtained through separation under a microscope. Composite samples of many hundreds of individuals were then rinsed with distilled deionized water and dried prior to analysis. Samples of *Diporeia hoyi* and oligocheates were obtained in benthic grab samples. Benthic samples were also stored in ethanol rinsed with distilled deionized water and dried prior to analysis. Ethanol preservation was found to remove isotopically light lipids, increasing $\delta^{13}C$ signatures by up to 1.5‰ (Leggett 1998).

The rates of areal primary productivity (PP, g $C \cdot m^{-2}$) reported here were determined utilizing ^{14}C uptake in shipboard incubators, as described in Millard et al. (1996). Monthly average values for PP integrated through the epilimnion were used in comparisons with the $\delta^{13}C$ values of POM (Table 1). The relative concentrations of $CO_{2(aq)}$ and HCO_3^- were calculated as described in Leggett (1998).

An Optima VG continuous-flow isotope ratio mass spectrometer (CF-IRMS) was used to obtain the $\delta^{13}C$ and $\delta^{15}N$ values for all of the particulate, zooplankton, and benthic samples. Instrument accuracy, precision, and range of linearity were monitored with sets of National Institute of Standards and Technology U.S.A. standards of known concentration and isotope composition including IAEA-CH6, IAEA-N1, and IAEA-N2. Duplicate standards of identical mass, within the linear range of operation, consistently had measured values within 0.25‰ of each other. All POM samples were treated with 2 N HCl to remove traces of $CaCO_3$. Subsamples of collected material had acid added to them; these samples were redried at 60°C and analyzed. This procedure was repeated until successive analyses were identical, demonstrating that all residual $CaCO_3$ had been removed.

Results

The $\delta^{13}C$ of DIC was observed to increase about 2.0‰ between April and October at both sites in each of the years

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Fig. 1. $\delta^{13}C$ of DIC in surface water at (A) Station 41 in 1994 (crosses) and 1995 (triangles) and (B) Station 81 in 1994 (crosses) and 1995 (triangles).

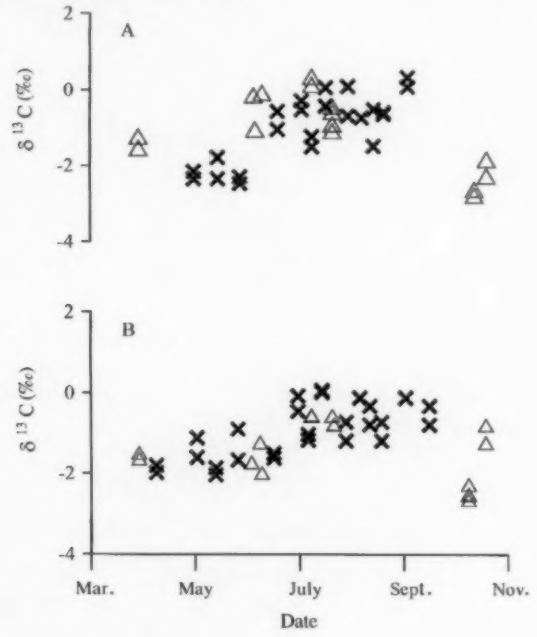


Fig. 2. Seasonal trends at Station 41 in 1995: (A) $\delta^{13}C$ of POM (1–20 μm size fraction) in relation to the $\delta^{13}C$ of different size fractions of zooplankton (crosses, >110 μm; circles, >210 μm; triangles, >295 μm, (B) surface water concentrations of Chl *a* and POC, and (C) surface water concentrations of HCO_3^- and $CO_{2(aq)}$.

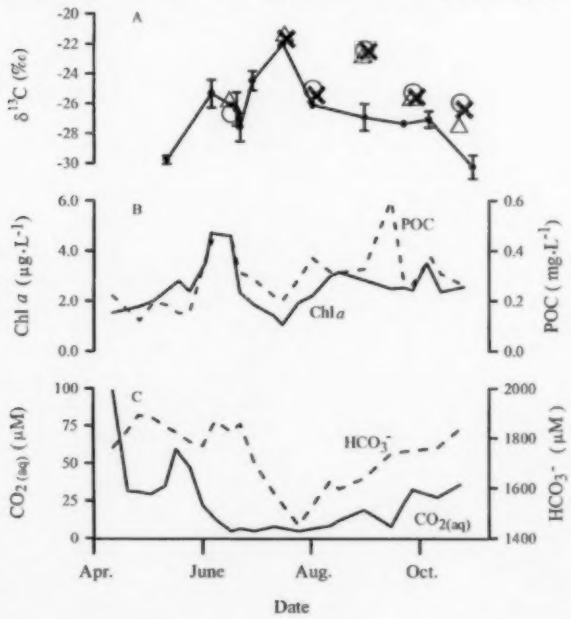
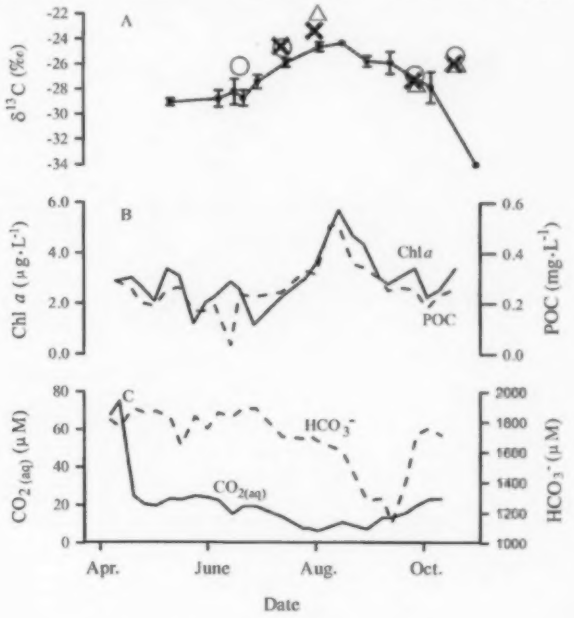


Fig. 3. Seasonal trends at Station 81, 1995: (A) $\delta^{13}C$ of POM (1–20 μm size fraction) in relation to the $\delta^{13}C$ of different size fractions of zooplankton (crosses, >110 μm; circles, >210 μm; triangles, >295 μm, (B) surface water concentrations of Chl *a* and POC, and (C) surface water concentrations of HCO_3^- and $CO_{2(aq)}$.



investigated (Fig. 1). There was no discernible diurnal fluctuation in the $\delta^{13}C$ of DIC. Duplicate samples, collected at increasing depths, indicated that the $\delta^{13}C$ of hypolimnetic DIC was about 1.5‰ lower than that found in the epilimnion (Leggett 1998).

A seasonal shift was observed in the isotope signatures of POM in the 1–20 μm size fraction collected on GF/F filters (Figs. 2 and 3). In late May through mid-June, the $\delta^{13}C$ of POM >20 μm in diameter was enriched in ^{13}C relative to all other size-classes of POM at both sites (Tables 2 and 3). The $\delta^{13}C$ of POM collected on filters (1–20 μm size fraction) at stations 41 and 81 increased about 6‰ from spring minimum values. Over the period in the fall when thermal stratification breaks down, the $\delta^{13}C$ of POM drops back down to under –30.0‰ at both sites. No correlation between the $\delta^{13}C$ of POM, Chl *a* concentrations, or the areal rates of PP could be determined at either site. There was a negative correlation ($r = -0.86$, $P = 0.002$, $\alpha = 0.05$, df = 10) between the $\delta^{13}C$ of POM and the concentration of $CO_{2(aq)}$ at Station 81 and between the $\delta^{13}C$ of POM and temperature ($r = 0.82$, $P = 0.006$). At Station 41, the $\delta^{13}C$ of POM was not significantly correlated with the concentration of $CO_{2(aq)}$, Chl *a*, or PP.

Differences in carbon signatures were observed depending on the size fraction of POM collected. Larger size fractions had a higher $\delta^{13}C$. The discrepancy between size fractions increased during the summer months (Tables 2 and 3).

The 110–210 μm fraction of zooplankton collected was made up of nauplii, smaller copepodids, rotifers, and some bosminids in different proportions, depending on the time of sampling. The 210–295 μm fraction contained bosminids, *Daphnia* spp., and calanoid and cyclopoid copepods includ-

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Table 1. Areal rates of photosynthesis (g $C \cdot m^{-2} \cdot month^{-1}$) at Stations 41 and 81 determined from the results of shipboard incubation of water samples with ^{14}C addition.

	Station 41		Station 81	
Time period	Empirical solar	Cloudless solar	Empirical solar	Cloudless solar
Season	132.8	181.3	151.1	193.9
Stratified period	84.0	115.9	107.6	136.5
May	27.1	35.7	21.6	27.3
June	24.7	33.0	20.7	25.3
July	17.9	25.8	25.1	31.7
Aug.	24.4	32.3	39.5	48.8
Sept.	24.6	33.2	25.6	33.1
Oct.	14.1	21.3	18.6	27.8

Note: The monthly PP rates were calculated for both empirical and theoretical cloudless (maximum) levels of solar irradiance. The stratified period for Station 41 was June 19 – October 16 and for Station 81 was June 24 – October 20.

ing *Diacyclops thomasi*. All of the size fractions contained trace amounts of phytoplankton that could not be removed by filtration (Figs. 2 and 3).

The $\delta^{13}C$ of zooplankton samples collected in the spring through early summer reflected the $\delta^{13}C$ of the POM collected on GF/F filters (Figs. 2 and 3). In late summer at Station 41, there was a 4‰ difference in $\delta^{13}C$ between the zooplankton samples collected and the 1–20 µm size fraction of POM (Fig. 2). The $\delta^{13}C$ of *Diporeia hoyi* was similar to the $\delta^{13}C$ of POM and zooplankton collected from the epilimnion in the spring (Table 4). Oligochaetes were enriched in ^{13}C relative to the *Diporeia hoyi* samples collected.

Discussion

The lack of correlation between PP and the $\delta^{13}C$ of POM on a seasonal basis suggests that photosynthetic uptake is not the sole determining factor of $\delta^{13}C$ of DIC and POM in Lake Ontario. The $\delta^{13}C$ of the POM collected also likely depends on the mode of uptake and source of carbon available to the algal cells present.

The observed 2.0‰ change in $\delta^{13}C$ of DIC in Lake Ontario (Fig. 1) is hypothesized to be a function of a shrinking pool of available $CO_{2(aq)}$, which is also becoming enriched in ^{13}C. The low level of seasonal change in the $\delta^{13}C$ of DIC, relative to the fluctuation observed in POM, may be attributed to the system's alkalinity and the magnitude of the pool of DIC available in Lake Ontario.

The measured carbon isotope signature of DIC is an integration of all of the carbon molecules making up the total pool of available DIC. As most of the DIC is HCO_3^-, the $\delta^{13}C$ of DIC is dominated by the $\delta^{13}C$ of HCO_3^-. This partially explains the observed seasonal stability of the $\delta^{13}C$ of DIC in relation to the $\delta^{13}C$ of POM. While the $\delta^{13}C$ of $CO_{2(aq)}$ and the $\delta^{13}C$ of HCO_3^- are both likely to shift on a seasonal basis, the $\delta^{13}C$ of HCO_3^- will be fairly stable, since concentrations of HCO_3^- remain relatively high. Therefore, a large amount of carbon has to be exchanged in order to get a shift in the $\delta^{13}C$ of the entire pool of DIC. In addition, the equilibrium between $CO_{2(aq)}$ and HCO_3^- is the predominant mechanism for exchange of carbon isotopes in the HCO_3^- pool. Therefore, changes in the signature of HCO_3^- have to be produced as a result of changes in the $\delta^{13}C$ of $CO_{2(aq)}$ and (or) changes in the fractionation associated with the equilibration of $CO_{2(aq)}$ and HCO_3^- (Mook et al. 1974; Marlier and O'Leary 1984). The $\delta^{13}C$ of $CO_{2(aq)}$ in the system is more susceptible to change because the concentration is smaller and there are a number of physical, chemical, and biological processes influencing both the concentration and the $\delta^{13}C$ of $CO_{2(aq)}$. It is predominantly $CO_{2(aq)}$ that is used as a substrate for photosynthesis, and therefore, shifts in the concentration and the $\delta^{13}C$ of $CO_{2(aq)}$ are reflected in the $\delta^{13}C$ of POM.

The temperature of the lake in April or early May is usually between 2 and 4°C, which is near the seasonal minimum (Johannsson et al. 1998). Colder winter temperatures enhance $CO_{2(aq)}$ solubility so that $CO_{2(aq)}$ contributes more substantially (5.3% as opposed to 0.3% midsummer) to a slightly larger DIC pool (about 1900 µmol $C \cdot L^{-1}$ as opposed to 1500 µmol $C \cdot L^{-1}$). The elevated concentration of $CO_{2(aq)}$ results in a DIC pool with a lower $\delta^{13}C$, since $CO_{2(aq)}$ is depleted in ^{13}C relative to HCO_3^-. The –2.2‰ signature for DIC measured in the spring represents a seasonal low.

The lake does not freeze over the winter and $CO_{2(aq)}$ is free to exchange with atmospheric CO_2. In the winter and early spring, Lake Ontario is supersaturated with CO_2 and acts as a source of CO_2 to the atmosphere, losing between 2 and 3 g $C \cdot m^{-2} \cdot day^{-1}$ (Weiler 1974). CO_2 exchange across the air–water interface will act to push the $\delta^{13}C$ of $CO_{2(aq)}$ toward –8.0‰, the value for the $\delta^{13}C$ of atmospheric CO_2 (Keeling et al. 1979). Given the net flux of CO_2 from the water column, it is unlikely that isotopic equilibrium exists between $CO_{2(aq)}$ and atmospheric CO_2.

The onset of spring and subsequent warmer temperatures coincides with elevated levels of primary production. The $CO_{2(aq)}$ pool is used preferentially as a source of carbon for primary producers, and concentrations of $CO_{2(aq)}$ start to fall. Isotopically light carbon is drawn out of the $CO_{2(aq)}$ pool as the heavier isotope of carbon is discriminated against by the enzyme systems active in carbon fixation. With rising temperatures and an increase in primary production, there is an increase in the pH of the system and a resulting decrease in the solubility of $CO_{2(aq)}$. By the time the lake is thermally stratified in mid-June, the concentration of $CO_{2(aq)}$ has dropped from >70 to <10 µM. Continued removal of $CO_{2(aq)}$ leads to a reversal in the exchange of CO_2 with the atmosphere as the system becomes a sink for atmospheric CO_2. The combination of these processes is at least partially re-

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Table 2. $\delta^{13}C$, $\delta^{15}N$ (±SD), and the C/N ratio of POM samples collected at Station 41 in 1995.

	20–44 µm				0.45–44 µm				1–20 µm				
Date	$\delta^{13}C$	$\delta^{15}N$	*n*	C/N	$\delta^{13}C$	$\delta^{15}N$	*n*	C/N	$\delta^{13}C$	*n*	$\delta^{15}N$	*n*	C/N
May 11		10.01			–28.4±2.38	7.18±0.83	9	5.51	–30.46±0.90	5	11.44±0.49	6	6.08
June 7	–23.11	3.39	2	9.67					–26.36±0.93	4	7.58±0.35	7	6.91
June 16									–26.25±0.09	3	4.18±0.57	3	8.77
June 19	–26.51±0.74	1.90±2.39	6	7.50	–29.4±0.95	3.70±0.66	7	6.62	–28.92±1.11	3	4.28	1	7.02
June 21	–26.90	0.71	1	7.72	–27.8±0.47	2.53±0.20	8	9.35	–27.57±0.97	3	3.51±0.19	3	7.97
June 29	–22.43	7.54	2	5.93					–24.49±0.65	4	4.59±0.59	7	7.17
July 15	–18.70	7.08	2						–23.86	2	5.55±0.26	4	9.85
Aug. 1	–21.85±2.22	8.49±1.66	3	8.73					–26.83	2	4.22±0.42	5	9.49
Aug. 30									–26.93±0.89	3	5.68	1	6.41
Sept. 21									–27.36	2	7.57±0.64	3	6.72
Oct. 5									–27.08±0.54	3	7.96±0.36	4	6.85
Oct. 23	–25.05	9.08	2										
Oct. 30									–30.26±0.78	4	10.02±0.63	5	7.95

Note: The samples were collected as follows: 20–44 µm, on Nitex screens; 0.45–44 µm, with a Westfalia centrifuge; 1–20 µm, on glass fibre filters after prefiltering through 44- and 20-µm-mesh screens.

Table 3. $\delta^{13}C$, $\delta^{15}N$ (±SD), and the C/N ratio of POM samples collected at Station 81 in 1995.

	20–44 µm				0.45–44 µm				1–20 µm				
Date	$\delta^{13}C$	$\delta^{15}N$	*n*	C/N	$\delta^{13}C$	$\delta^{15}N$	*n*	C/N	$\delta^{13}C$	*n*	$\delta^{15}N$	*n*	C/N
May 10					–29.3±0.76	4.41±0.59	9	7.16	–29.01±0.28	5	4.73±0.26	5	9.13
June 6									–28.73±0.66	3	8.53±0.63	3	7.68
June 15									–28.18±1.02	4	5.01±0.30	3	8.98
June 20	–27.40	4.77	2	10	–29.4±0.77	4.01±0.46	11	7.98	–28.67±0.62	3	3.54±0.32	3	6.62
June 28									–27.38±0.54	3	4.52±0.30	3	7.55
July 14									–25.83±0.33	3	4.29±0.81	6	8.21
Aug. 2	–19.03	6.36	2	11					–26.50±0.71	3	5.23±0.75	6	8.26
Aug. 15									–24.28	2			
Aug. 29									–25.71±0.42	3	7.26±0.42	4	7.49
Sept. 11									–25.83±0.88	3			
Oct. 4									–27.79±1.24	3	7.81±0.28	3	6.38
Oct. 20	–25.17±0.73	8.31	3	5									
Oct. 30									–31.72	2	11.84	1	8.23

Note: The samples were collected as follows: 20–44 µm, on Nitex screens; 0.45–44 µm, with a Westfalia centrifuge; 1–20 µm, on glass fibre filters after prefiltering through 44- and 20-µm-mesh screens.

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Table 4. $\delta^{13}C$ of individual species of zooplankton and benthic invertebrates collected at Station 41.

Date	*Diacyclops thomasi*		Calanoid copepods		*Bosmina* spp.		*Daphnia* spp.		*Diporeia hoyi*		Oligocheates	
	$\delta^{13}C$	*n*	$\delta^{13}C$	*n*	$\delta^{13}C$	*n*	$\delta^{13}C$	*n*	$\delta^{13}C$	*n*	$\delta^{13}C$	*n*
1994												
Apr. 20									–28.45	8	–23.68	2
May 12	–27.63	2	–26.28	1								
May 26	–28.01	2	–26.39	1								
June 6	–27.60	1	–26.58	2					–26.61	3	–24.54	2
June 30	–25.15	5	–26.48	1	–24.43	2						
July 14	–24.23	1			–22.89	2			–26.95	7		
July 28	–21.67	1			–22.93	1						
Aug. 11	–22.69	2			–22.42	2	–23.49	1	–27.21	2	–22.58	4
Aug. 29	–21.78	3	–24.36	2	–23.18	4						
Sept. 27	–23.64	2										
Oct. 6	–21.60	1	–22.85	1	–22.76	2	–24.40	1				
Oct. 13			–23.01	3			–22.99	1	–27.15	6	–24.18	3
1995												
May 11	–28.52	2	–29.07	2								
June 16	–25.34	6	–25.15	1	–25.47	2						
June 21	–25.05	1	–24.91	1	–26.04	2						
June 29					–25.38	1						
Aug. 1	–23.58	2	–24.95	1	–24.44	3	–25.17	1				
Aug. 30	–21.11	1										
Sept. 26	–22.81	1	–29.00	1	–24.49	1	–24.01	1				
Oct. 23			–27.11	1	–24.48	1	–25.93	1				

Note: All samples are composites. Zooplankton was sampled from 20 m to the surface when the lake was mixed and from the midepilimnion to the surface when the lake was thermally stratified.

sponsible for the observed increase in the $\delta^{13}C$ of DIC in the epilimnion, from –2.2 to –0.5‰, where it remains for much of the summer. As the $CO_{2(aq)}$ pool shrinks and becomes more enriched in ^{13}C, the process of equilibration between $CO_{2(aq)}$ and HCO_3^- becomes faster, as there is less carbon in the $CO_{2(aq)}$ pool to turnover and less total DIC in the system. The isotopic equilibrium, such that it exists, is shifted and subsequently, both $CO_{2(aq)}$ and HCO_3^- become more isotopically enriched. At higher temperatures, the carbon isotope fractionation of $CO_{2(aq)}$, with respect to dissolved HCO_3^-, is less pronounced (–8.97‰ at 25°C) (Mook et al. 1974). In addition to the biological and chemical kinetic influences, the influx of atmospheric CO_2 maintains the low levels of $CO_{2(aq)}$ at near-equilibrium concentrations (Boston et al. 1989). The concentration of CO_2 added from the atmosphere is larger than that from epilimnetic respiration and the entire pool is circulated very rapidly. Therefore, this source may also serve to stabilize the $\delta^{13}C$ of $CO_{2\ (aq)}$ at about –8.0‰, the air–water equilibrium value. Under conditions where there is an influx of atmospheric CO_2, the $\delta^{13}C$ of POM is not as susceptible to enrichment by primary producers, since the concentration of $CO_{2(aq)}$ is maintained by a source of carbon that is isotopically light relative to HCO_3^-.

In the hypolimnion, where primary production is not a significant factor influencing the DIC pool, respired CO_2 from sediment/fauna and colder temperatures maintain a DIC pool that is depleted in ^{13}C relative to epilimnetic DIC. There is little or no mixing of hypolimnetic and epilimnetic $CO_{2(aq)}$ so that atmospheric CO_2 is not a mitigating factor. When the lake turns over in the fall, there is mixing of epilimnetic and hypolimnetic DIC. This addition of ^{13}C-depleted DIC, along with cooler fall temperatures and $CaCO_3$ precipitation, boosts the concentration of $CO_{2(aq)}$ available to primary producers and results in a decline in the carbon isotopic signatures of DIC and POM. The observed decline would be expected to continue into the winter until the lake again reaches a point where the it attains a balance and a resulting $\delta^{13}C$ signature of about –2.2‰ for DIC.

The high $\delta^{13}C$ of POM while the lake is stratified can most easily be attributed to an increase in the $\delta^{13}C$ of $CO_{2(aq)}$ and (or) a reduction in the level of isotope fractionation by primary producers due to reduced concentrations of available $CO_{2(aq)}$. Alternatively, the elevated signatures may be due to the active uptake of DIC or the facilitated conversion of HCO_3^- to $CO_{2(aq)}$ by primary producers (Kaplan 1985). These mechanisms are most likely to be active under conditions of $CO_{2(aq)}$ limitation, since the active uptake of HCO_3^-, or the facilitated conversion of HCO_3^- to $CO_{2(aq)}$, has an attached energetic cost that must be overcome before any net benefit is derived (Raven and Lucas 1985). The concentrations of $CO_{2(aq)}$ in Lake Ontario are probably sufficient for diffusive uptake of CO_2 to meet the carbon requirements for a majority of the primary producers (Leggett 1998). However, it is possible that some species may find it advantageous to use HCO_3^- to meet their photosynthetic demands. For lakes of high alkalinity (1.5–3.0 mequiv.·L^{-1}), such as Lake Ontario, the pH range 8.1–8.3 also represents an air-equilibrium state. As a result, these lakes may be more susceptible to pH elevation and CO_2 depletion (Talling 1985).

The direct assimilation of HCO_3^- by Lake Ontario picoplankton has previously been observed in ^{14}C addition experiments (Caron et al. 1985). Boundary layer effects,

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limiting $CO_{2(aq)}$ at the cell surface, or low CO_2 to O_2 ratios in the cell may also induce some species of primary producers to use compensating mechanisms for carbon uptake (Prins and Elzenga 1989; Tsuzuki and Miyachi 1989). This may explain some of the variability in the $\delta^{13}C$ of different size fractions of POM collected. The observed differences are possibly a function of species-specific differences in uptake and fractionation of carbon isotopes. The sample may include algae using HCO_3^- as a carbon source. Hodell and Schelske (1998) noted the high concentrations of choococcoid cyanobacteria in Lake Ontario in late summer as potentially having a role in calcite formation. *Synechococcus* can use HCO_3^- as a source of carbon-producing hydroxyl ions, which could induce calcite formation.

The enriched $\delta^{13}C$ of the zooplankton collected in late summer at Station 41 suggests a food source other than the POM collected on the filters. This could reflect feeding on algae that are using an enriched source of carbon such as HCO_3^-. Alternatively, the elevated zooplankton signatures in late summer may be a lag from the elevated signatures of POM generated earlier in the season. The carbon from POM recycled through bacteria and mesotrophic algae feeding on bacteria may remain in the system for a longer period of time. Sources of organic carbon (particulate organic carbon (POC)) other than photosynthetic algae, indicated by the concentration of Chl *a*, are elevated in late summer and may play a significant role in sustaining secondary production in the system (Fig. 2).

The $\delta^{13}C$ signatures of the *Diporeia hoyi* collected at Station 41 indicate the importance of production early in the seasonal cycle in sustaining benthic primary consumers (Table 4). *Diporeia hoyi* will feed on POM at the sediment surface, and their $\delta^{13}C$ will reflect the $\delta^{13}C$ of the bulk of the POM available. Signatures of *Diporeia hoyi* increase slightly as the season progresses but remain consistently below values for epilimnetic POM, produced after the lake has stratified. In contrast, the oligochaetes collected had a higher $\delta^{13}C$ than most of the POM samples measured in this study. Oligochaetes feed on bacteria and organic detritus below the sediment surface: the original source of organic carbon is probably identical to that consumed by *Diporeia hoyi*. Therefore, the difference between the *Diporeia hoyi* and oligochaete signatures may be attributed to diagenesis of surficial sediment. Alternatively, the discrepancy may be an indication of different dietary sources of carbon.

Conclusions

The results of this study suggest that a seasonal fluctuation in the $\delta^{13}C$ of POM is largely influenced by the algal response to the ambient concentration of $CO_{2(aq)}$ and also that the $\delta^{13}C$ of POM may not necessarily be an effective indicator of productivity in all circumstances. There are a number of processes influencing the $\delta^{13}C$ of DIC and POM at any point in the season. The $\delta^{13}C$ observed depends on which process is dominant at any given time. Any relationship between the $\delta^{13}C$ of POM, levels of productivity, Chl *a*, or the $\delta^{13}C$ of DIC is likely to be system and season specific depending on the processes controlling $CO_{2(aq)}$. The range in $\delta^{13}C$ of different size-classes of POM at different times also suggests a species-specific relationship between the $\delta^{13}C$, availability of $CO_{2(aq)}$, and the $\delta^{13}C$ of the organic carbon produced. Therefore, using the $\delta^{13}C$ of organic carbon in sediment may not always be a robust means of inferring past productivity in lacustrine systems.

An understanding of system biogeochemistry and potential for change and variability in the $\delta^{13}C$ of primary producers should be an intrinsic part of any study where carbon isotopes are used to make inferences regarding carbon source in aquatic systems. In this study, the $\delta^{13}C$ of primary consumers reflected the source of carbon available to them at any particular point in time. Characterization of the seasonal pattern in POM therefore provided some insight into the relative importance of primary production at different times during the season to benthic and pelagic primary consumers. Since a number of factors influence the carbon signature of POM on a temporal basis, and are not likely to be consistent between systems, it is difficult to make inferences as to the food source of organisms without characterizing the variability in potential food sources.

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DISCUSSION

Comment: Fatty acid signatures and classification trees: new tools for investigating the foraging ecology of seals[1]

Otto Grahl-Nielsen

The composition of blubber of marine mammals has been the goal of a large number of investigations during the last 70–80 years (Ackman and Lamothe 1989). Differences in the fatty acid composition have been detected among different species of seals and whales, even among different populations within species and between pups and adults. It is the prevailing assumption that these differences are caused by differences in diets.

The continuing development of analytical equipment has increased the sensitivity and resolution of the analyses, allowing detection and quantification of 70 or more fatty acids in samples of blubber, or any other tissue, from marine animals. With such complex mixtures, evaluation of the data calls for a multivariate approach where the information contained in all fatty acids is retrieved simultaneously (Kaufman 1992). Nevertheless, in the vast number of published investigations on lipids, univariate methods, e.g., Student's *t* test, have been, and still are, the predominant way of data handling. However, multivariate applications on fatty acid data appear with increasing freqeuncy in the literature. The first applications were on terrestrial samples, where classification of olive oils from different geographical regions has dominated (Forina and Armanio 1982; Forina and Tiscornia 1982; Derde et al. 1984). Classification of bacteria on the basis of their content of fatty acids is also a typical multivariate approach, applied even earlier than classification of olive oils (Jantzen et al. 1974). In fact, a commercialized package for identification of bacterial species based on multivariate computations of fatty acids has been developed (microbial identification system (MIDI), Microbial ID Inc., Newark, Del.).

An extensive number of applications of multivariate evaluation of fatty acid profiles have been carried out during the last 10 years, i.e., characterization of moulds (Blomquist et al. 1992), taxonomy of marine fungi (Cooney et al. 1993), taxonomic studies on spruce (Grahl-Nielsen et al. 1991), characterization of beef fat tissue (Gaydou et al. 1984), and distinction among lavandula hybrids (Maffei and Peracino 1993).

Multivariate applications on marine fatty acids first appeared in the last part of the 1980s (Grahl-Nielsen and Barnung 1985; Vogt et al. 1986; Ulvund and Grahl-Nielsen 1988). The most common multivariate method is principal components analysis (PCA). A more advanced form of PCA, soft independent modelling of class analogy (SIMCA), has also found widespread use. A related method is linear discriminant analysis (LDA), while cluster analysis is used in some instances.

The common feature of all the multivariate methods is that they treat all the variables simultaneously by placing the samples in a multidimensional space with one dimension for each of the measured variables. Before this is done, the analytical data are usually preprocessed by scaling and (or) transformation. The goal is then to group the samples according to similarity, which is associated with their position in the multidimensional coordinate system. The various methods differ in the manner that the distances among the samples are mesasured and how they are grouped.

The distances may be computed in the original coordinate system, as in cluster analysis. Most methods do, however, generate new coordinates, which describe the optimal distribution, with regard to a priory defined criteria, of the samples in the original coordinate system. Similarities, or differences, among the samples are then evaluated in the new coordinate system. In the case of PCA, the new coordinates are called principal components, and these are placed in the directions of the largest, second largest, etc., spread of the samples, without considering possible relationships among the samples. This is a nonsupervised method. In the case of LDA, the new coordinates are called canonical variates. They are generated in such a way that the best discrimination among given groups of samples in the data set is obtained, i.e., this is a supervised method (Massart and Kaufman 1983).

Another difference between LDA and PCA is that the number of variables has to be much less than the number of samples in LDA. In modern analytical chemistry, much data are generated, and the number of variables often exceeds the number of samples. This is of no concern in PCA (Wold 1979). However, the number of principal components that

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can be computed is limited to the number of samples in the cases where the number of samples is lower than the number of variables. This may lead to the misunderstanding that the number of variables has to be restricted to the number of samples. In fact, in the advanced application of PCA, SIMCA, the modelling of the classes is improved with increasing numbers of variables. This is a supervised method, where geometrical bodies, structured on principal components, encompass the samples that belong together in classes. The method allows the user to control whether the samples really belong to a class, whether they are outliers, or whether they belong to another class. The distances of all samples from the various classes may be determined, as well as the distances, i.e., similarities, among the classes.

At present, the most active group in marine mammal lipid research is S. Iverson's group at Dalhousie University, Halifax, N.S., Canada. They have produced a wealth of data on several seal species such hooded seals (Iverson et al. 1995), Antarctic fur seals (Iverson et al. 1997*a*), and harbor seals (Iverson et al. 1997*b*), as well as on black bears (Iverson and Oftedal 1992). For handling of these data, they have advocated the use of classification and regression trees (CART). Their latest publication (Smith et al. 1997) is dedicated to the effort of showing the usefulness of this statistical method as a tool in studies of foraging ecology of seals. In this respect, they attempt to demonstrate the superiority of the classification tree method over PCA, with extensive referencing to Grahl-Nielsen and Mjaavatten's (1991) use of PCA. This has prompted the present discussion. I will discuss both the general use of CART in classification of this type of data and the way in which Smith et al. (1997) apply the method to investigate the foraging ecology of seals.

The CART method is a supervised, stepwise method with very good classification power. On the basis of a training set with known class allocations of the samples, all variables are first tested for their deviation among the classes. The variable with the highest deviance, a predictor variable, is then used to divide the samples into two groups where the value of that variable is below a certain cutoff value in one group and above the cutoff value in the other group. The procedure is repeated for each of the two groups by detecting other predictor variables and their cutoff values. In this manner, an inverted tree is obtained and the splitting continues until the branches contain samples of a single class only.

The CART method has been developed fairly recently by statisticians (Breiman at al. 1984) and is practically unknown in the chemometrics community. Frank and Lanteri (1989) compared it with discriminant analysis, linear and quadratic, and with PCA in the form of SIMCA. CART was found to perform as well as the other methods for classification purposes, but it was found to be superior with respect to simplicity and interpretability. The simplicity of the method is demonstrated by the fact that test samples can be classified manually when a classification tree is established on the basis of a training set.

This is because the strategy of the method is a sequence of univariate evaluations. Thus, CART is not a true multivariate method, in that the information is not withdrawn from a combination of the variables; in other words, the samples are not placed into a multidimensional coordinate system.

All samples in a class do not necessarily have values of a predictor variable that place them on the same side of the cutoff point in the node. Therefore, samples that actually belong to the same class are passed down different branches of the classification tree. In this manner, samples from the same class will end up in two or more end nodes (Frank and Lanteri 1989; Iverson et al. 1997*a*, 1997*b*; Smith et al. 1997). This is of no concern for classification purposes: a test sample will be correctly classified as long as it ends up in one of the end nodes that is allocated to that class. For example, judged by the rate of misclassifications, CART performs well in comparison with other classification methods (Frank and Lanteri 1989; Smith et al. 1997).

Although performing well for classification purposes, a serious drawback with CART is that no information about the relationship among classes of samples is obtained. This is obvious in all applications of CART referred to in this discussion, where samples from one class appear in two or more end nodes on completely different branches, well apart from each other on the classification tree. This lack of information about relationship is further demonstrated in the paper of Smith et al. (1997). Here, the four classes of milk samples would be expected to appear in succession, since they have a continous change in the composition of the fatty acids, as judged from the table of relative amounts given in the paper. There is, however, no systematics in their appearance in the branches of the classification tree.

Another consequence of the missing ability to give information about relationships is that the method gives no indication of where misclassified samples may actually belong. On the other hand, the true multivariate methods, PCA, SIMCA, LDA, and cluster analysis, where the classification is based on combinations of the variables, give results, in the form of displays or computed distances, that, in addition to the class allocations, give information about distances, i.e., relationships, among groups, or among groups and samples, or among samples. A further consequence of CART's missing ability to determine the relationship among the groups of samples is, of course, that the causes for any differences among groups cannot be found.

However, PCA, as a true multivariate method, will perform all these tasks. This is demonstrated here by a PCA of the data of Smith et al. (1997, their table 1) (Fig. 1). The milk samples appear in succession from day 0 to days 19–21, as expected from their table 1. When the data in their table 1 are computed without any transformation, only the fatty acids present in the largest amounts are of importance for the distinction of the samples (Fig 1*a*), i.e., 18:1*n*9, 20:1*n*9, 22:1*n*11, and 20:1*n*11, positioned to the right of the origin, increase from day 0 to days 19–21, while 20:5*n*3, 16:0, and 14:0 decrease from day 0 on. The fatty acids 22:6*n*3, 16:1*n*7, and 18:1*n*11 are also of importance for the difference among the samples, but they are located further from the origin along the PC2 axis than the former fatty acids. This is because they do not have a continuous change from day 0 to days 19–21, and this causes a curved distribution of the samples in the plot. Thus, recomputation without 22:6*n*3 results in a straighter distribution of the samples (Fig. 1*b*), and the variation along PC2 decreases from 10 to 4%, while the variation along PC1 increases from 88 to

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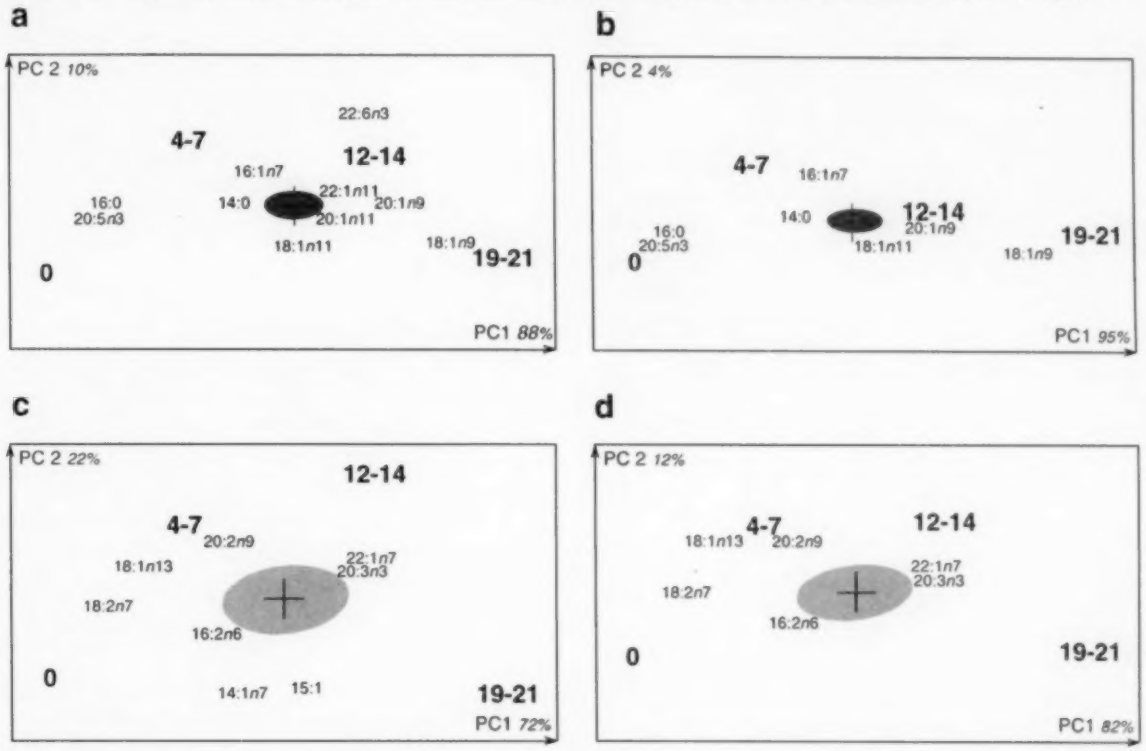
Fig. 1. PC1–PC2 plots based on PCA of the data presented in Smith et al.'s (1997) table 1. The seal milk samples were collected on the day of parturition (0) and at 4–7, 12–14, and 19–21 days postpartum. (The four samples used here are averages of between nine and 15 individual samples.) The measured variables are 64 fatty acids, and their amounts, as a percentage of the total, are used in the computations. (*a* and *b*) Data used directly; in Fig. 1*b*, the computations are done without 22:6*n*3. (*c* and *d*) Data logarithmically transformed; in Fig. 1*d*, the computations are done without 14:1*n*7 and 15:1. The fatty acids with the largest importance for the position of the samples are shown in the plots (see text); the others are located in the shaded area around the origin in the plots.



95%. All the other fatty acids, present in smaller amounts, are located close to the origin in the plot, within the shaded area, and have little importance for the spread of the samples.

To evaluate if any information is contained in the fatty acids present in smaller amounts, the differences in magnitude of the concentrations were levelled out by taking the logarithm, and a new PCA was carried out. This resulted in the same curved distribution of the samples with about the same distances between them (Fig. 1*c*), but now, another set of fatty acids, i.e., those that have the highest relative difference among the samples, shows importance for the distribution of the samples.

Two fatty acids, 14:1*n*7 and 15:1, present in minor amounts, are the ones most responsible for the variation along the second principal component, i.e., for the curved distribution of the samples. Recomputation without them increased the variation along PC1 from 72 to 82%, while the variation along PC2 was reduced from 22 to 12% (Fig. 1*d*).

It is also noticable that the fatty acid 16:2*n*6, which in CART was found to have the largest deviance and therefore was chosen as the predictor variable for the first node (Smith et al. 1997), ranks well behind 18:2*n*7 and 18:1*n*13 in importance for the distribution of the samples. It is seen in Smith et al.'s (1997) table 1 that the relative difference from day 0 through days 19–21 is larger for the two latter fatty acids than for 16:2*n*6. In fact, 16:2*n*6 does not have a continous change from day 0 through days 19–21. As many as 20 fatty acids, out of the 64 given in their table 1, do have a continous change through the lactation period.

Due to this continous change, the PCA shows an approximately even distribution among the four sampling times, both in the case where the fatty acids present in the largest amounts are decisive (Figs. 1*a* and 1*b*) and in the case where those fatty acids present in smaller amounts have been given relatively higher importance by logarithmic transformation (Figs. 1*c* and 1*d*). Contrary to this, Smith et al. (1997) interpreted their CART results as giving a more difficult distinction between the two last sampling periods than among the other samples, while they, on the other hand, found a "steady increase" in several fatty acids over the sampling period.

It is demonstrated here that PCA shows relationships among the samples that comply with the analytical data and that information of how the variables, i.e., fatty acids, influence this relationship is obtained. It is also demonstrated that PCA works perfectly well in cases where the number of variables exceeds the number of samples, 64 variables versus four samples here.

The lack of these properties in CART is a serious draw-

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back, since in studies of foraging ecology of seals, the strategy is to relate differences in the fatty acid composition of blubber, or milk, among seals feeding on different prey items to differences in the fatty acid composition of possible prey. However, Iverson's group never used CART to compare blubber, or milk, with prey. After having classified the samples from seals, e.g., after lactation periods (Iverson et al. 1997*a*; Smith et al. 1997) or by geographical area (Iverson et al. 1997*b*), using CART on the whole set of analysed fatty acids, they selected only a few of those fatty acids that fit with the fatty acid composition of the prey that they assumed the seals had eaten.

They assumed, without documentation, that the diving female harbor seals from Sable Island feed predominantly on northern sand lance (*Ammodytes dubius*) throughout the lactation period. Then, they selected three fatty acids, 20:1*n*9, 22:1*n*11, and 24:1*n*9, that increase steadily in the milk over the lactation period, ignoring the other seven fatty acids with the same increasing trend and the 10 fatty acids with a decresing trend, and since these three acids have "generally high levels" in northern sand lance (here, too, documentation is lacking), they used this as an indication that their assumption was correct.

The same approach was used in an investigation on the diet of lactating Antarctic fur seals (Iverson et al. 1997*a*), although in this case the change in abundance of the few selected fatty acids did not even fit into their conclusions. Milk samples from four stages of lactation were first classified by CART on the basis of 47 fatty acids. As usual, samples belonging to one group were devided into several groups, i.e., the 11 samples from the midforaging period fell into three separate groups. A change in the relative amount of three of the 47 fatty acids was used to suggest that the seals changed from teleost fish in their prepartum diet to krill at the onset of the foraging trips and back to teleost fish during mid- to late foraging trips. Although they were unable to analyse directly the fatty acid composition of krill and fish, they supported their conclusions on literature data, stating that "high levels of 18:1*n*9, 20:1*n*9, and 22:1*n*11 tend to be characteristic of many teleost fish, not of krill." A decrease in these three fatty acids from the perinatal milk samples towards the milk samples from the early and midforaging period followed by an increase in the late foraging period should then be expected. However, this was only the case for 20:1*n*9, while 22:1*n*11 did not fall from the perinatal to the early and midforaging period, and 18:1*n*9 fell through all foraging periods.

Despite the above critical comments, it cannot be ruled out that a change in diet will result in a change in the fatty acid composition of the milk or blubber. However, to determine the magnitude and character of such an influence, and to later be able to apply it in foraging studies, will require very well controlled experiments. A good approach could be to use captive seals. The turnover rate of the lipids from the diet as well as the condition of the seals have to be taken into consideration. In the case of blubber, the large differences through the depth of the blubber has to be taken into account. In comparisons of the fatty acid composition in milk and blubber with that of the diet, a powerful multivariate program is mandatory, since small changes have to be detected on top of profound differences between diet and milk or blubber. For this purpose, CART is certainly not suitable.

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Reply: Fatty acid signatures and classification trees: new tools for investigating the foraging ecology of seals[1]

Stephen J. Smith, Sara J. Iverson, and W.D. Bowen

It is unfortunate that the comment by Dr. Grahl-Nielsen is based on a misinterpretation of our use of classification and regression trees (CART), a misinterpretation of our discussion of their merits, and an erroneous application of principle components analysis (PCA) to our example data. Herein, we address the issues brought up in his comment.

CART has been advanced as a useful way of classifying data based on a number of variables. Indeed, as Grahl-Nielsen's comment acknowledges, CART's classification power has been demonstrated previously and has been found to perform as well as discriminant analysis and PCA, but with the added advantages of simplicity and ease of interpretation. The purpose of our paper (Smith et al. 1997) was to illustrate the features and value of CART in the analysis of fatty acid data and to compare these results with other types of multivariate analyses. For this purpose, we used milk samples collected throughout the lactation period from female harbour seals. As stated in our paper, since female harbour seals fast during the first 4–7 days of lactation, relying solely on blubber stores for milk production, followed by feeding trips (Boness et al. 1994), we hypothesized that we might see changes in milk fatty acids over time and therefore could use fatty acids as a tool to indicate changes in foraging patterns (e.g., Iverson 1993; Iverson and Oftedal 1995). We chose classification trees because unlike some of the standard multivariate methods (linear discriminant analysis, PCA, etc.), one can screen all 65 measured fatty acids with this method, even when their number exceeds the number of seals sampled. Grahl-Neilsen has taken issue with our approach and has proposed that PCA is applicable in this situation, does not suffer from needing more observations than variables, and provides a more meaningful analysis.

In our study, a PCA would proceed as follows. For each female seal in the study, we sample its milk and measure p fatty acids. Given a sample of n seals, we would have an $n \times p$ matrix of observations. A PCA would aim to reduce the p dimension of this matrix to q dimensions ($q << p$) via linear combinations (usually) such that combination i explains the ith largest portion of the total response variance of the observations. In order to do this, $n \geq p$ and the covariance or correlation matrix of the observations is used. The general result is that we would have a small number of principal components that explain most of the variability in the data. Plotting the scores for the first principal component (which will be a linear combination of all fatty acids for each seal) against the second principal component for each animal, we would look for some natural clustering of points that may reflect postparturition periods. This method is exploratory at best and requires that we have more seals than fatty acids.

Grahl-Nielsen states that PCA can be used even when the number of fatty acids exceeds the number of observations. Legendre and Legendre (1998) listed this situation in their "Misuses of principal components" subsection. Application of PCA to the covariance or correlation matrix assumes that we have a statistically valid estimate of this matrix, which is only possible when $n \geq p$ (also see Ripley 1996, p. 289).

Grahl-Nielsen chooses to show that PCA would work for our data by first using the data in our table 1 (Smith et al. 1997) where mean fatty acid compositions for all seals within a time period postparturition are presented for each fatty acid. By using mean compositions, Grahl-Nielson ignores individual variation in fatty acid composition. Next, he applies a PCA to these data where it appears, given that individual fatty acids are labelled on his fig. 1, that the fatty acids are treated as observations and time periods are treated as variables (hence, in this case, $n > p$). Our original problem was, given a fatty acid signature at any one time, could we tell how many days postparturition the female's milk was collected. However, Grahl-Nielsen has twisted the problem to be that, given a vector of compositions over the time periods for a given fatty acid, we might be able to tell what fatty acid it was or what group it may belong to. That is, we do not seem to be working on the same problem. Furthermore, the problem analyzed by Grahl-Neilsen is of doubtful biological interest.

Grahl-Neilsen states that the classification tree method gives no information of where misclassified samples may actually belong. Given that we have to know the original classification of the seals to build the tree, then we do know where the misclassified samples actually belong. However, if his point was that because we only show the predicted class for each seal, we do not know how reliable the prediction is, then, again, he is incorrect because there is information available in CART that is analogous to distances in the

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Table 1. Posterior probabilities from a pruned classification tree (Smith et al. 1997, fig. 4) for misclassified specimens.

	Posterior probability				Class	
Specimen	Day 0	Days 4–7	Days 12–14	Days 19–21	Predicted	Actual
M6MK14	0.00	0.00	0.40	0.60	Days 19–21	Days 12–14
M7MK14	0.00	0.00	0.40	0.60	Days 19–21	Days 12–14
M8MK07	0.00	0.18	0.82	0.00	Days 12–14	Days 4–7
M9MKNB	0.07	0.86	0.07	0.00	Days 4–7	Day 0
M10MK04	0.93	0.07	0.00	0.00	Day 0	Days 4–7
M12MK07	0.00	0.18	0.82	0.00	Days 12–14	Days 4–7
M13MK14	0.07	0.86	0.07	0.00	Days 4–7	Days 12–14

Note: Predicted class is given by the highest posterior probability.

multivariate techniques listed by Grahl-Neilson. For each seal, the classification tree model predicts the probability that the seal is a member of each of the classes (or terminal nodes). The class with the largest probability is assigned as the predicted class. Consider these probabilities for the seven misclassified samples from the harbour seal milk data using the pruned tree in fig. 4 of Smith et al. (1997). The model prediction for the first two specimens appears to be less certain than for the remaining five and may suggest that the former group of specimens may be transitional in some sense (Table 1). While the actual class is known for these specimens, this kind of information would be useful for judging the certainty of predictions even when the class is unknown.

The rest of Grahl-Nielsen's comment dwells on how much better his analysis is and on how we claimed things that were completely unfounded. He states that we tried to demonstrate the superiority of CART over PCA. We did no such thing. We only suggested that CART was an additional tool with some attractive features for these kinds of data. He then goes on to state that CART is not an appropriate tool in studies of foraging ecology because it cannot be used to predict the composition of diets. Unfortunately, Grahl-Nielsen seems not to appreciate that there are several ways of studying foraging ecology. One way is to ask if fatty acid signatures of one group of animals differ from those of another in time or space. CART seems to be quite a valuable tool in answering this type of question (Iverson et al. 1997*a*, 1997*b*; Smith et al. 1997). However, contrary to Grahl-Nielsen's claim, we have never suggested that a CART analysis of predator fatty acids could be used to estimate the composition of a predator's diet. Neither CART nor PCA could be used for such a purpose. To suggest this seems quite naive. The problem of quantitatively relating predator fatty acids to diet is a far more complex issue. Currently, research is underway at Dalhousie University on the development of statistical models that address this more complex problem (e.g., Iverson et al. 1999).

Grahl-Nielsen goes on to quite erroneously imply that we concluded based on selected fatty acids that the seals used in our test data were eating northern sand lance. We made no such conclusion. We did point out that northern sand lance is the major prey of lactating females at Sable Island. This conclusion is based on lavage samples of the females' stomachs during the breeding season, as we stated in the paper. We then only observed, as a point of discussion, that the increase in several fatty acids known to be abundant in northern sand lance "may reflect...northern sandlance" in the diet. Perhaps Grahl-Nielsen did not understand this point in our paper. The same was true in discussing various aspects of fatty acid changes in Antarctic fur seals (Iverson et al. 1997*a*).

Finally, Grahl-Nielsen ends by pointing out that controlled feeding studies on captive seals will be necessary to validate the use of fatty acid signatures in estimating the species composition of diets. Certainly this is true. Perhaps this statement is with the recognition that his captive study (Grahl-Nielsen and Mjaavatten 1991) was inconclusive. This study reported on sampling of the outermost 1–2 cm (an area least affected by diet) of blubber from only one grey seal pup and one harbour seal fed unknown rations and analyzing only four homogenates of herring (12 individuals) and four individual mackerel throughout the course of the 7-month study, thus precluding any assessment of true diet effects or variability in seals or their prey. Thus, well-designed captive feeding studies are clearly needed. Such experiments are currently underway in our laboratory (S.J. Iverson and W.D. Bowen) as well as in others. After all of this, classification trees and PCA remain exactly as they were: different and complementary means for classifying and differentiating samples based on numerous variables. Beyond this, it is difficult to understand the point of Dr. Grahl-Nielsen's comment.

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ERRATUM

Erratum: Nitrogen–phosphorus relationship in high mountain lakes: effects of the size of catchment basins

Rafael Morales-Baquero, Presentación Carrillo, Isabel Reche, and Pedro Sánchez-Castillo

Ref: Can. J. Fish. Aquat. Sci. **56**: 1809–1817 (1999).

On p. 1809, in the second-last line of the right-hand column, "positive" should replace "negative" to read:

"a positive relationship between this ratio and catchment area".

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Rafael Morales-Baquero, Presentatción Carrillo, Isabel Reche, and Pedro Sánchez-Castillo	**2227**	Erratum: Nitrogen–phosphorus relationship in high mountain lakes: effects of the size of catchment basins

I.K. Birtwell, R. Fink, D. Brand, R. Alexander, and C.D. McAllister **2087–2098**
Survival of pink salmon (*Oncorhynchus gorbuscha*) fry to adulthood following a 10-day exposure to the aromatic hydrocarbon water-soluble fraction of crude oil and release to the Pacific Ocean

Erling Otterlei, Gunnar Nyhammer, Arild Folkvord, and Sigurd O. Stefansson **2099–2111**
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